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PREFACE.

Since the occupation of the Netherlands in May 1940 postal exchange with foreign countries, especially those overseas, has gradually decreased and broke down completely in 1941, thereby depriving us of the opportunity to keep in touch with our colleagues abroad by exchangeing periodicals and reprints of papers in our scientific field, as in every other branch of science.

Happily many of our colleagues after the war have been quick to provide us with their publications issued in the past few years and the Board of the Netherlands Society of Microbiology is glad in its turn to seize the opportunity of reciprocity by collecting in one volume abstracts of all papers on research work in the field of microbiology, serology and related sciences from periodicals issued in the Netherlands during the years 1940—1945, both in dutch and foreign languages. In the medical field papers dealing mainly with pathology and epidemiology are omitted.

It is hoped that the exhange of papers from 1946 onwards will follow its normal course as in the years before the war, thereby reestablishing the many friendly relations which existed between dutch microbiologists and their foreign colleagues.

The abstracts are arranged according to subjects. The main classification is:

General microbiology
Biochemical investigations
Serological investigations
Medical bacteriology and serology
Animal pathology
Technical microbiology
Mycology and plant pathology
Soil bacteriology

We owe a dept of gratitude to the staff of our periodical Antonie van Leeuwenhoek for the considerable amount of work involved in preparing this issue.

The Netherlands Society of Microbiology,

JAN SMIT, Chairman

IN MEMORIAM PROF. G. KAPSENBERG

November 9th 1942 G. KAPSENBERG, Professor in Hygiene and Medical Police at the University of Groningen passed away quite unexpectedly.

GERARDUS KAPSENBERG was borne April 24th 1883 at Rotterdam. After having passed the High School at The Hague he studied medicine at the University of Leiden. On April 8th 1909 he received his medical certificate. Already as a student he showed his ability, *e.g.*, by the treating of a subject proposed for prize competition by the Medical Faculty at Leiden. His paper bore the title: „An experimental and clinical investigation of the reaction of the organism on the products which the decay of the cells of its own organs give rise to". The Senate valued this investigation with the golden medal, accorded in a public meeting in 1909.

The extensive scientific interests of KAPSENBERG were evidenced by his fulfilling the function of demonstrator in various branches, before as well as after having attained his medical certificate. First he was demonstrator to R. P VAN CALCAR, Professor of Hygiene. Then during two years he received a surgical training under the direction of Prof. J. A. KORTEWEG. In 1911 he returned to Prof. VAN CALCAR for a short period and from Oct. 1st 1912 to Dec. 1st 1917 he was demonstrator and later head demonstrator to Dr. D. A. DE JONG, professor of Comparative Pathology. Moreover he practised in surgery as well as in bacteriology and sérology! During the first world war KAPSENBERG occupied several other governmental functions, *viz.*, surgeon in the military medical Service, adviser for hygiene of the city of Leiden, controlling medical officer of the food department and school doctor.

In 1919 he was appointed as Director of the newly established service of Hygiene at Groningen which had been completely organised by him. And as the culmination of his scientific career after the death of ALEX. KLEIN he was appointed as Professor of the University of Groningen in January 1936. On June 4th 1936 he held his inaugural address on „Microbiology and immunology as a science."

The scientific work of KAPSENBERG as well bears evidence of his many sidedness. First of all his publications in the immunological field draw the attention, which bear chiefly on the relation of the antibodies of the serum globulines and which culminate in an interesting study on the formation of antibodies.

Next to this his work in the field of bacteriological diagnostics is of chief interest. As early as 1913 and 1919 he published some papers on the technique of the Wassermann reaction. In 1922 he thrice detected *Microsporum pubescens flavescens* thus far never detected in the Netherlands. Further he was the first in the Nether-

lands to isolate the bacillus of Cohen as the causal agent of meningitis (1924). A still rarer bacterium, *viz.*, *Listerella monocytogenes*, detected in 1926 in infections of rabbits, was isolated by KAPSENBERG for the first time from man, also in a case of meningitis. A detailed investigation of bacteriological diagnostics of diphtheria (1932), in which he improved the nutrient medium by the addition of egg's yolk and glucose, gives evidence of his lasting interest in routine tests.

The epidemiology of infectious diseases as well has been enriched by his work. This is evidenced by a study of meningitis cerebrospinalis (1918), a report on the danger of contagion in tuberculosis composed together with G. J. HUET, by descriptions of minor epidemics of typhoid and paratyphoid fever at Groningen (1920, 1924) and by his observations of the plague flea *Ceratophyllus fasciatus* at Groningen (1926). Even in the surgical field he worked scientifically. A paper on a hip trouble published in 1916 bears witness thereof.

In all of his work the technical side of our difficult branch of science had his special interest. He imagined continuously technical improvements or new apparatus, which he knew how to realize with great dexterity. His papers on methods of dialysis by means of amnion membranes, on a metal dop as a substitute for the cotton plug and on new apparatus for filtration, elution and extraction bear witness of the important results arrived at. KAPSENBERG was an excellent speaker and it was always a treat to follow his carefully prepared courses and lectures. But most of all he was a sincere and open-hearted man, kind-hearted and ready to help. Thus his sudden passing away means a great void for his many friends, co-workers and pupils. They will not forget what he did for them.

A. E. B.

IN MEMORIAM Dr H. A. DIDDENS

Many of her friends in Holland and also abroad will have heard with deep sorrow of the sudden death of MANNIE DIDDENS during the war. She died the third of December 1944 at Amersfoort. She met with an accident, misled by the darkness and died immediately after.

She had a very active life and the work she had already achieved offered so many expectations for her future. It seems incredible that such a valuable life has ended now for ever.

HERMANNA ANTONIA DIDDENS was born in February 8, 1902 at Winschoten, where she attended the elementary school and Latin school. In 1921 she entered the University of Amsterdam as a student in biology. She passed her final examination in general botany and zoology, entomology, phytopathology and microbiology in 1937 and continued to work on phytopathology at Professor WESTERDIJK's Institute at Baarn. She took her doctors degree on a thesis entitled: ,,Investigations on a flax disease caused by *Pythium megalacanthum* de Bary'' at Amsterdam, in 1931.

She had interrupted her study at Baarn, however, for about a year to work as a demonstrator in phytopathology at Professor SCHAFFNIT's Laboratory at Bonn.

When she still worked for her thesis she was, in April 1929 appointed as collaborator at the ,,Centraalbureau voor Schimmelcultures'' where she remained till September 1942. Her chief work at the ,,Bureau'' was the control of the collection and the indentification of the numerous cultures sent to the ,,Bureau''. Moreover she did the extensive correspondence. Besides this work she largerly contributed to a study on the systematics of the *Mycotoruloideae*. In 1932 she spent, in connection with this work some time at the Institute of Parasitology of Dr. LANGERON at Paris.

She showed a deep interest in work going on in other laboratories and in methods used there. This interest connected with a great passion for travelling made her visit many laboratories and institutes during her numerous journeys in Germany, England, France, Italy, Denmark.

In 1936 she attended the second- and in 1939 the third international Congress for Microbiology resp. at London and at New York. At the latter she read a paper entitled: ,,Variations occuring in type culture collections''. After the New York Congress she visited many laboratories and institutes in the eastern part of the U.S.A. She stayed for some time at the Institute of Dr. WAKSMAN at New Brunswick (N.J.) and at Dr. WESTON's Institute at Cambridge (Mass.). The war menacing her own country made her return to Holland at the beginning of 1940.

In 1942 she changed her position at the ,,Centraalbureau''

for another, which offered her better prospects. She, then, was appointed as a mycologist at the ,,Laboratory for Bulbresearch" at Lisse. Her work there, more in the field of phytopathology, had her highest interest. Unfortunately war-conditions very soon made scientific work almost impossible. To the ,,Centraalbureau voor Schimmelcultures" the departure of its able collaborator was a very great and deeply felt loss.

She had been secretary of the Dutch Society for Plantpathology. During this period she published — in 1941 — together with T. A. C. SCHOEVERS and H. L. G. DE BRUYN a list of Dutch names for plant diseases in agricultural plants. Her other publications were mainly about the work going on at the ,,Centraalbureau" and several of them were published in this periodical.

She was a very talented scientist and an accurate researcher. But besides the deep interest she showed in her work, she also had an open eye for other things. She was very fond of sports, of music and other branches of art. She had all kind of favourite pursuits *e.g.*, she studied Russian for some time and had made such progress in this language that she succeeded together with a fellow pupil to translate a russian scientific book.

As a schoolgirl she was president of the club of grammar-school pupils and as a student she was member of the board of the corporation of students.

She liked domestic comfort and was an excellent housewife, and the pleasant years we lived together in the little house ,,Madoera" at Baarn I will always remember as a good time. Her chief trait of character was her perfect honesty as well in scientific as in human respects. This together with her great modesty, her cheerfulness, her warm feeling towards others and her keen sense of humour made the friendship with her of so great a value.

Therefore the tragic end of her life will be both a loss to science and a grief to her many friends, who will always remember her peculiar charm and vital personality.

J. L.

IN MEMORIAM PROF. Dr W. C. DE GRAAFF

After a long illness passed away on May 10, 1944 Dr W. C. DE GRAAFF, Professor in Pharmacography, Galenic Pharmacy and Applied Biology at the University of Utrecht and Honorary Doctor of the University of Amsterdam. As intensive as his interest in many branches of science as extensive were his knowledge and the field he covered in his teaching. In fact he taught pharmacography as well as the science of foodstuffs, of medicinal, nutritional and toxic plants, the medical chemistry and applied microbiology. As to his lectures they were always clear and captivating, enlivened by his humour and irony.

When merely his microbiological work will be mentioned here, this does not mean that he would have been less active in other fields, far from it. It may be stated, however, that microbiology stood first in his interest. This is evidenced by the large number (20) of theses on microbiological subjects which have been composed under his direction.

Next to the strictly scientific microbiology applied microbiology had his interest. The important work performed by him first as member and later as president of the Committee ex Art. 17 of the Wares Act needs mentioning. In this function he did not keep to his official instruction, but, and this the more so when the bacteriological condition of victuals or stimulants was in question, he arrived at his opinion by means of personal investigations, an opinion which generally would further find its way into the Royal Warrants. Also the establishment of the Normal Leaf N 1028 bearing on the bacteriological examination of drinking water is due to his initiative and has been accomplished under his direction after a period of about 9 years, wherein many investigations bearing on this matter had been carried out in his laboratory.

In the more strictly scientific field he became known by his investigations of the bacterial fermentation, where he attempted to attain insight in the fermentation of sugar by means of fermentation of simpler compounds. As micro-organisms he studied mainly coliform bacteria, which have always roused his special interest.

In his laboratory for the first time in the Netherlands the antigenic structure of bacteria has been investigated, *viz.*, of various types of pneumobacilli.

Already from his Leiden period dated his interest in the methodics of clinical bacteriological research, which was evidenced in the book he wrote together with Prof. Dr E. GORTER, *viz.*, „Klinische Diagnostiek" a widely valued laboratory manual.

Until his death he was president of the Committee for the examination of clinical analysts, instituted by the Chemical Society. He has always acted vigorously for a thorough training of these auxillary laboratory workers.

IN MEMORIAM PROF. Dr P. C. FLU

On December 19, 1945 the University of Leiden as well as the Institution for Tropical Medicine had to encash a heavy loss by the passing away of PAUL CHRISTIAAN FLU. Not merely as a very outstanding scientist but as well as organiser and leader of this Laboratory he was to be valued greatly. He was always ready for his coworkers and if one had the privilege of composing a thesis under his direction, one might be sure that Professor FLU would assist with word and deed and when needed would give actual personal help. Also for the lower staff working under his direction the death of this beloved chief means a heavy loss. To his great simplicity and to the interest he took in their difficulties it is due that they not merely lose a Director but even more a counsellor upon whom they knew to may depend.

Professor FLU was born in 1884 in Paramaribo where he was trained as a West-Indian physician. By his outstanding qualities he drew the attention in such measure that he was among those who might finish their studies in the Netherlands. At the age of hardly 22 he obtained in 1906 at Utrecht his certificate as a physician. After a period of one year in which he was demonstrator of Prof. SPRONCK he was appointed as a military surgeon second class in the army of the Netherland East Indies. In order to increase as much as possible his medical knowledge he worked first some years at Paris and Hamburg. In the latter city he worked in the Institution for Naval and Tropical Diseases, where Dr VON PROWAREK directed his studies. In this Institutions his first publications in the parasitological field saw the light.

This period of life came to a close in 1908 when he had to follow his destination for the Military Hospital at Paramaribo. Here he was put to work in the pathological laboratory. An order to investigate scientifically the occurrence of malaria in the upper lands of Suriname followed in 1910. Professor FLU teaching meanwhile in the Medical School as well, it needs no further stress that in this period of 1908 to 1910 an enormous amount of work was performed. As the result thereof he wrote some very important communications which were mainly published in foreign periodicals. These papers, which up till now have not in the least lost in value, have highly contributed to the drawing of the attention on this sturdy scientific worker. More and more he appeared to be of great value in the furthering of the knowledge of tropical disease. It is him that Netherland East and West Indies may thank for important observations which have promoted public health in a marked degree.

On July 24, 1911 he was appointed once more in the Netherland East Indies where a further development of his scientific aptitude was opened to him, when he had to replace the fourth medical officer in the medical laboratory at Weltevreden. In 1915 after the leaving of Dr G. GRIJNS his appointment as Director followed. In the mean time Prof. FLU taught general parasitology in the Indonesian Medical School, thus the amount of work performed here did not remain under the level attained at in his West Indian period.

Notwithstanding this he had always a ready ear for us younger physicians, who gladly came to him with our problems. For many of us he has thus been of great value.

His gifts of leadership did not remain in the dark. In 1921 the chair for Tropical Hygiene at Leiden was offered to him and June 11, 1921 he held here his inaugural address on the subject: The influence of factors outside the human organism and the spreading of infectious diseases in the tropics.

Having initially worked in a fairly primitive laboratory on the Keizersgracht at Leiden, it was a great satisfaction for Professor FLU to be able to move over to the laboratory on the Rapenburg, arranged according to his directions. At the same time he was appointed as Director of this tasteful and well equipped Institution for Tropical Hygiene.

In his further scientific investigations he centred mainly on one subject, *viz.*, the bacteriophages, following on a period of intimate co-operation with D'HÉRELLE. In his research work Professor FLU assumed the help of many younger workers which gave rise to various Doctor's theses.

In the latter 25 years of his life he has kept to this subject nearly uninterruptedly. A short interruption of these activities occurred, when during 4 months he travelled for study in Suriname charged by the „Vereeniging voor Tropische Hygiëne".

Once again a change would be brought about in the branches of science taught by Professor FLU, when in 1936 as the result of a concentration of chairs he was charged with the teaching of Hygiene and Bacteriology.

Meanwhile many distinctions fell to his part. He was Knight of the Order of Oranje Nassau met de Zwaarden and Commander in the order of the Nile, whilst the University of Utrecht conferred upon him a Honorary Doctor's degree. Besides he was a corresponding member of the Royal Academy of Science at Amsterdam and of the „Vereeniging tot Bevordering der Geneeskundige Wetenschappen in Indië" and finally of the „Société de Pathologie exotique" and of the Belgian „Vereeniging voor Tropische Geneeskunde".

In 1938 Professor FLU was called upon as Rector Magnificus of the University of Leiden. Up till then the line of his life had tended upward. Alas, in that same year 1938 he became a victim of his work as by a laboratory accident he incurred a severe in-

fection. After having got through a serious illness, it initially appeared as if it would turn out for the good, but gradually it became apparent that his health had suffered greatly. Still Professor Flu untiredly kept on working at his favourite subject, the bacteriophage, wherein the problem of vaccination against plague had taken up an important part.

But fate did not favour him any more. Heavy afflictions were bestowed upon him, which finally were too great for his decreased corporal resistance. Still he kept on fighting up till the moment that the summarising of the knowledge of the bacteriophage during the latter 25 years had been brought to a close. This work will be published ere long.

Among his further larger publications we cite: The textbook of parasitary diseases and of Hygiene (3 volumes), Textbook of Tropical Hygiene, his report of his visit of Suriname and the Chapter „Die Pest" in Mense's Handbuch der Tropenkrankheiten and finally „Voordrachten over de Aetiologie en Prophylaxe van Infectieziekten", the re-edition of which will be issued ere long, which has still been supervised by Professor Flu. A. P.

GENERAL MICROBIOLOGY

Verzamelde Geschriften van M. W. BEIJERINCK. Zesde deel. Met registers op alle zes deelen benevens eene beschrijving van zijn leven en beschouwingen over zijn werk door G. VAN ITERSON Jr., L. E. DEN DOOREN DE JONG en A. J. KLUYVER. (Collected papers of M. W. BEIJERINCK. Sixth volume. With indices on the six volumes and a description of his work by G. VAN ITERSON Jr., L. E. DEN DOOREN DE JONG and A. J. KLUYVER). Martinus Nijhoff, den Haag, 1940.

The sixth volume has been published 20 years after the initial 5 volumes. Next to some scientific papers published by BEIJERINCK after he had left the chair at Delft Volume 6 contains 3 indices (Author Index; Index to organisms; Subject Index) which are a great help to the reader, who wants to get the most out of the wide variety of subjects masterfully treated by BEIJERINCK. The chief value, however, lies in the three treatises in the English language, composed by his successors in the chair and by his last pupil and demonstrator. VAN ITERSON has treated the botanical part of the work of BEIJERINCK, KLUYVER the microbiological part. By these most competent workers in their fields the work of BEIJERINCK has been discussed in the light of present-day knowledge and the products of BEIJERINCK's creative imagination can stand this light. DEN DOOREN DE JONG treats the scientist from the human side. He has composed a biography and has drawn a portrait of BEIJERINCK as a scientific worker. He has succeeded in drawing a portrait very true to life and although a fervent admirer of BEIJERINCK he has been honest enough not to leave out the shade parts. In the chapter „BEIJERINCK at work" a very vivid picture is presented of the scientist, of his sparkling vitality and his characteristic habits and sayings.

The sixth volume rounds up the impressive work of the five earlier very worthily.

L. E. DEN DOOREN DE JONG, De voedingswaarde der bacteriën in verband met hun pathogeniteit. (Nutritive conditions of bacteria in connection with their pathogenicity). Chemisch Weekblad **29**, 131, 1942. Cf. Over de evolutie der bacteriën in verband met het ontstaan der pathogeniteit. (On the evolution of bacteria in connection with the origin of the pathogenicity). Ned. T. voor Geneeskunde **86**, 734, 1942. Cf. also: Een en ander over bacterieele groeistoffen. (Notes on bacterial growth substances). Vakblad voor Biologen **23**, 38, 1942.

The pathogenicity of bacteria, when seen from the standpoint of the evolutionary doctrine, is an acquired property, only developed

after the existence of higher plants and animals had become possible and after bacteria had succeeded in conquering the natural resistance of organisms. According to KNIGHT five evolutionary stages can be noted among bacteria: 1. Autotrophic bacteria. 2. Heterothropic bacteria. 3. Bacteria requiring amino-acids as N-source. 4. Bacteria which need growth-substances in addition to the organic C- and N-compounds. 5. Bacteria which can develop exclusively in animal organisms. Among bacteria of group 1 and 2 no real pathogenic forms occur, most of them belonging to group 4. Transitions are found between the different stages. The synthetic ability decreases from 1 to 5. The pathogenicity of bacteria is caused by their forming endo- or exo-toxines. The question arises why a saprophytic bacterium may have started to develop pathogenic properties. Comparing pathogenic bacteria with saprophytic bacteria of the same genus it becomes evident that — without exception — the pathogenic put much more complicated demands on nutrition. With the increasing of pathogenicity the bacteria have lost the ability of synthesising certain substances themselves. On the other hand, cultivation on artificial media may decrease the pathogenic ability and increase the enzymatic potencies. (*E. typhosa*, *M. tuberculosis*).

It is now supposed that pathogenic bacteria have developed out of saprophytes because through the stay in rich nutritive surroundings the synthetic ability is decreased. The bacteria are now forming toxic substances to attack living tissue in order by these means to get hold of the necessary nutritive substances.

P. C. FLU, Das Ultravirus als Krankheitsursache, seine Eigenschaften und eine kritische Uebersicht über die Ansichten bezüglich seiner Art. (The ultravirus as a pathogene, its characteristics and a critical survey of the existing conceptions as to its nature). Acta Leidensia (Mededeelingen uit het Instituut voor Tropische Hygiëne, Leiden) **15—16**, 25, 1940—1941.

The author gives a clear survey of the characteristics of ultravirus (generally including phage also in this term) as far as they are founded on experiments. These well-founded facts are: The whole group of agents which are embraced by the term „ultravirus" are not uniform. The smallest and most primitive among them, such as virus diseases of plants, the phages and viruses of many human and animal diseases — as far as they have been studied — consist of nucleoproteids, which, when purified as far as possible, sometimes have been obtained as paracrystals. The higher organised agents such as the elementary bodies of vaccine and of variola moreover consist of a fat soluble substance and carbohydrates. In accordance with their dimensions which oscillate between 10—150 $\mu\mu$ they follow the laws of the hydrophile colloidal state of matter. To develop their functions they need an equilibrium of

electrolytes in the same way as living protoplasm. Whenever this equilibrium is destroyed, they are rendered inactive or they are annihilitated. All these characteristics are characteristics of living matter.

The author discusses critically the various theories brought forward as to the nature of ultravirus, and more in detail the theory of endogenous origin and that of dead matter. The author claims that virus, phage and ultravirus exist in the region on the borderline of living and dead matter and it seems to him wise to acknowledge here the same mystery which is adherent to all living matter.

P. C. Flu, Worden na contact met een bacteriophaag megatherium niet-lysogene stammen lysogeen, omdat zij met den phaag worden geïnfecteerd, of omdat zij tot phaagvorming worden geinduceerd? (Do non-lysogene strains after contact with a bacteriophage megatherium grow lysogene, because they are infected with the phage or because they are induced into phage formation?) Acta Leidensia (Mededeelingen uit het Instituut voor Tropische Geneeskunde, Leiden) **15—16**, 52, 1940—1941.

The lysogene *Bacillus megatherium* 338 which, in ordinary circumstances, does not produce phage, when cultivated in peptone-water, produces the same phage that dissolved it and rendered it resistent and lysogene. The probability that this faculty is due to phages that penetrated into its plasma and later were enclosed in the spores, is greater than that it has assumed the property by some mysterious induction.

S. J. C. Dunlop, Investigations on the occurrence of bacteriophage-free spores of *Bacillus megatherium* 899 of den Dooren de Jong. Antonie van Leeuwenhoek **7**, 234, 1941.

It has been tried to isolate from *B. megatherium*, strain 899 of den Dooren de Jong a spore that does not contain phage. This has failed. Moreover it has been proved, that in case a suspension containing spores is heated at 80° C., not all the spores are resistant to this temperature as one would suspect, but only about 1 %. It is left open whether phage-free spores may occur.

T. Y. Kingma Boltjes, Some experiments with blown glasses. Antonie van Leeuwenhoek **7**, 61, 1941.

Experiments were carried out with blown glass spheres in order to ascertain whether the observations reported by van Leeuwenhoek might be made by means of these. Microphotographs made through such lenses show clearly that this is actually the case. van Leeuwenhoek, however, mentions his use of ground lenses. At low magnification these are certainly better than blown glasses.

The possibility of VAN LEEUWENHOEK having used coverslips is discussed.

A. E. BEUTE, Méthode simple pour la dessiccation dans le vide de cultures bactériennes. (Simple method for the drying in vacuo of bacterial cultures). Antonie van Leeuwenhoek **10**, 71, 1944—1945.

The apparatus used is a modification of the model of COOPER and GRABELL. A simple and rapid method for the drying of bacterial cultures has been arrived at.

P. H. H. DE BRUYN, The cultivation of filterable viruses in vitro. Antonie van Leeuwenhoek **8**, 19, 1942.

A review of the recent methods for cultivation of viruses on tissue cultures and cell suspensions is presented. The aims which may be arrived at by these mains are discussed.

A. Bos, Het desinfecteerend vermogen van kresyline op enkele bacterie-soorten. (The disinfecting action of kresyline on some species of bacteria). Tijdschrift voor Diergeneeskunde **70**, 55, 1943.

The disinfecting effect of kresyline upon *Esche ichia coli*, *Brucella abortus*, *Erysipelothrix rhusiopathiae*, *Shigella equuli*, *Salmonella enteritidis* var. *dublin*, *Staphylococcus aureus* and *Streptococcus pyosepticus* (syn. *Str. pyogenes*) appeared to be equal or better than that of creolin, and surpassed that of carbolic acid. These bacteria are killed by a 1 % solution within 2 minutes. In fact the effect on the germs of black leg and anthrax was better than that of creolin or citopogen, but distinctly less than that of carbolic acid. A 10 % solution of kresyline killed *Clostridium chauvoei* within 30 minutes, anthrax within 2 days.

C. F. VAN OYEN, Eenige onderzoekingen omtrent de bacteriedoodende werking der Westinghouse-Sterilamp. (Some investigations of the bactericidal action of the Westinghouse-Sterilamp). Tijdschrift voor Diergeneeskunde **67**, 586, 1940.

Irradiation with the Westinghouse-Sterilamp kills a good deal of micrococci, *B. coli* and Salmonellae in a thin layer of agar. The effect depends on the length of time of irradiation, the temperature and the distance between lamp and the layer of agar.

BIOCHEMICAL INVESTIGATIONS

A. J. KLUYVER and M. TH. J. CUSTERS, The suitability of disaccharides as respiration and assimilation substrates of yeasts which do not ferment these sugars. Antonie van Leeuwenhoek **6**, 121, 1939—1940.

Yeast species claimed as non-fermenting disaccharides were studied as to their ability to ferment maltose, lactose or saccharose. Non-maltose and non-lactose fermenting yeasts gave completely negative results. In tests for the fermentation of saccharose *Torula monosa* was quite negative, in 7 other species, however, a weak yet unmistakable fermentation could be observed. Heavy inoculations had to be applied and sometimes the time of observation had to be prolonged.

The assimilation was tested by means of the auxanographic method of BEIJERINCK and by growth experiments in liquid media with quantitative determination of the disappearance of sugar. The non-fermenting species *Torulopsis dattila*, *Torulopsis utilis*, *Mycocandida parakrusei* and *Brettanomyces anomalus* gave positive results. *Saccharomyces fragilis*, *Torula cremoris* and *Torula monosa* did not consume any maltose under anaerobic conditions. The consumption by *Zygosaccharomyces Marxianus*, *Saccharomyces exiguus* and *Saccharomyces Ludwigii* was so low that it may be practically neglected. Among the non-lactose fermenting yeasts *Blastodendrion intermedium* consumed a considerable amount of lactose, whilst *Saccharomyces carlsbergensis* and *Saccharomyces cerevisae* did not consume any. All non-saccharose fermenting yeast species with the exception of *Torula monosa* consumed saccharose under anaerobic conditions.

The suitability of the disaccharides as a respiration substrate has been tested by means of the WARBURG manometer method. Discrimination between respiration of sugar and of accompanying impurities has been obtained by extending the experiment over a longer period of time. All yeasts which had shown positive results in the fermentation test with maltose were able to use it as a respiration substrate. None of the non-lactose fermenting yeasts could use lactose as such. The non-saccharose fermenting yeasts with the exception of *Torula monosa* could use saccharose as a respiration substrate.

For *Torulopsis dattila* it could be proven that an aerobic fermentation exists along with the respiration. When the respiration is inhibited by cystein, this results in an immediate occurrence of aerobic fermentation. Thus a hydrolase is present in this yeast. Maltase was detected as well by means of the method of WILLSTÄTTER, SCHNEIDER and BAMANN. Moreover in *Mycocandida parakrusei*, *Saccharomyces italica* and *Schizosaccharomyces octosporus* the presence of saccharase could be proven.

Some unexplained anomalies still existing in the behaviour of the yeast, it has been tried, whether adaptation might influence

them. By cultivating *Blastodendrion intermedium* on a lactose medium, respiration of lactose was obtained.

It is discussed that apparently hydrolases are partially or completely inactivated under anaerobic conditions. Probably it is the state of reduction which will entail this inactivation. Proteinases are activated by reduction and it may be conceived that this increased proteolysis will affect the carbohydrases which contain proteins as main components.

M. Th. J. Custers, Onderzoekingen over het gistgeslacht *Brettanomyces*. (Investigations of the yeast genus *Brettanomyces*). Thesis, Delft, 1940.

17 strains of *Brettanomyces* yeasts were studied as to their morphological and physiological characters. Cells of a particular „ogive" shape frequently occur. All strains produce marked quantities of acid from glucose. *Brettanomyces* Kufferath et van Laer is to be maintained as a separate genus in the subfamily *Mycotoruloideae*. For the moment four species and two varieties should be distinguished within the genus.

Under anaerobic conditions ethyl alcohol and CO_2 were the only dissimilatory products formed by *Brettanomyces Claussenii*. Under aerobic conditions *Brettanomyces Clausenii* and *Brettanomyces bruxellensis* produced a considerable amount of acetic acid besides ethyl alcohol and CO_2. It is deemed probable that the acetic acid is a product of the oxidation of the ethyl alcohol. Under similar conditions *Saccharomyces cerevisiae* did not produce any acetic acid. In manometric respiration experiments it was found that *Brettanomyces Claussenii* oxidises the alcohol to acetic acid only at pH = 6.40, whilst at pH = 4.35 and 3.77 this acetic acid is further oxidised to CO_2 and water.

Manometric experiments were made to study the influence of O_2 on the fermentation of glucose. An early decline is apparent and caused by the formation of acetic acid from the accumulated ethyl alcohol. The most remarkable result was that the aerobic fermentation surpassed the anaerobic fermentation. When using cells of older cultures higher values of both aerobic and anaerobic fermentation were observed and these cells also show a normal Pasteur-effect. Cells derived from anaerobic cultures were characterized by a very low value of respiration, whilst the intensities of both aerobic and anaerobic fermentation were high and almost equal. A discussion has been given of the causes underlying the negative Pasteur-effect.

E. van Olden, Manometric investigations on bacterial denitrification. Proc. Kon. Ned. Akad. van Wet. **43**, 635, 1940.

The aim of the investigation was the testing of the possibility

of studying denitrification of „resting bacteria" by means of the manometric method. It was found necessary to use cells which had grown anaerobically in the presence of nitrate. Then in the manometric experiments an evolution of nitrogen could be observed of the same order of magnitude as that of the aerobic gaseous metabolism. The rate of nitrogen production proved constant as long as a sufficient amount of nitrate was present. Thus no intermediate stages of the nitrate reduction were formed in any appreciable amounts. The total amount of nitrogen evolved corresponded with the amount calculated when the nitrate added was completely converted. Thus the manometric method appears suitable for the study of denitrification.

Certain bacteria appeared to denitrify almost equally well in the presence as in the absence of organic substrates. So these are able to oxidize reserve material present in the cells at the expense of the nitrate added. This process has been termed „endogenous denitrification".

The previous history of the cells shows a decisive influence on their denitrifying activity. Recently arguments have been given in favour of the view that denitrification is depending on a special „nitrate reductase". This has to be considered then as an „adaptive enzyme".

H. Beeuwkes, Sur les ferments protéolytiques du *Vibrio cholerae* et du *Vibrio El Tor*. (On the proteolytic enzymes of *Vibrio cholerae* and *Vibrio El Tor*). Antonie van Leeuwenhoek **6**, **48**, 1939—1940; Cf. also: Acta Leidensia **15—16**, 134, 1940—1941.

Haemodigestion and the liquefying of gelatine are caused by separate enzymes. In 4 strains of *V. El Tor* cultivated on agar the splitting up of gelatine, estimated by means of the viscosimeter of Ostwald, and the haemodigestion were more intense than in strains kept on white of egg. In the latter the haemodigestive action had quite disappeared.

S. de Boer, Nitrate assimilation of *Aspergillus niger* van Tieghem. Proc. Kon. Ned. Akad. van Wet. **43**, 715, 1940.

The xylenol method for determining the nitrate in fungi and their culture solutions yields satisfactory results. Nitrate is not accumulated in *Aspergillus niger* but is metabolised after uptake. In the case of starved fungous mats the pH does not appear to have any effect upon the assimilation of nitrate. In growing fungi the assimilation of nitrate is strongest at pH 4, corresponding with the strongest development. Nitrate assimilation is increased by addition of glucose.

A. Gorter, Amino acid breakdown by *Aspergillus niger*. Proc. Kon. Ned. Akad. van Wet. **43**, 721, 1940.

The various enzymes hitherto known for the amino acid breakdown play no part in the deamination of amino acids by starved mats of *Aspergillus niger*. In this organism the oxidative deamination of amino acids proceeds best at pH 2—4 of the surrounding medium. It is closely coupled with the ordinary cell respiration, and is probably caused by ,,unspecific" oxidation enzymes.

L. H. C. Perquin, On the incidental occurrence of rod-shaped, dextran producing bacteria in a. beet sugar factory. Antonie van Leeuwenhoek **6**, 227, 1939—1940.

From a ,,Froschlaich" formed in a sugar-factory, the responsible organism was isolated. This organism could be identified with the dextran-forming, heterofermentative, rod-shaped lactic acid bacterium, *Betabacterium vermiforme*, a bacterium first described by Ward as one of the constituents of the ,,ginger-beer plant" and recognized by Mayer as being also one of the components of the ,,tibi"-consortium. Thus it has been shown that the formation of the ,,Froschlaich" in sugar-factories is not always due to *Leuconostoc mesenterioides* or to *Bacillus vulgatus*, but can also be formed by *Betabacterium vermiforme*.

A hitherto unknown species of the genus *Streptobacterium* was found to be an accompanying organism. This rod-shaped, homofermentative, dextran-forming bacterium has been described in detail as the species *Streptobacterium dextranicum* nov. spec.

F. A. M. J. Smits van Waesberghe, Onderzoekingen over microben-amylasen. (Investigations of microbial amylases). Thesis Delft, 1941.

The aims of the investigations reported on in this thesis consist in: 1°. the studying of the occurrence of amylolytic properties in various yeast species, including a more detailed study of the amylase occurring in *Saccharomyces fragilis*; and 2°. gaining an insight in the action of the bacterial amylases which occur nowadays on the market and a thorough investigation of the products of hydrolysis of starch produced by the latter.

The investigation as to the occurrence of amylases in 64 yeast species belonging to widely varying genera has shown that by far the greater number of these possessed a definite faculty to attack starch more or less intensively. This amylolytic faculty was distributed quite erratically over the various yeast species and showed important differences in the way in which the starch was attacked. In this connection the author compared the various methods by means of which the decomposition of starch might be ascertained

qualitatively. It could be proven that some species of *Hansenula* and *Saccharomyces* in pure cultures could ferment part of the starch, with the production of carbon dioxide and alcohol. It appeared that the amylolytic properties of *Saccharomyces fragilis* Jörgensen could be wholly attributed to phosphorylase. The preparation of amylase derived from bacteria, such as Superclastase, Rapidase, Biolase N-extra is discussed. All these preparations appeared to be free from maltase. The occurrence of glucose and maltose could be ascertained with certainty as products of hydrolysis induced by Superclastase. Moreover a polysaccharide fermentable by *Saccharomyces cerevisiae* could be isolated and identified as a trisaccharide.

J. H. Bekker, Les mutilats du bacille charbonneux. (The mutilates of *Bacillus anthracis*). Antonie van Leeuwenhoek **7**, 180, 1941.

For 11 strains of *Bacillus anthracis* it has been investigated whether they were rendered asporogenous by incubating at 42° C. Some strains never lose their sporogenous character, other are asporogenous for a period, but soon grow sporogenous again and finally there are some strains which remain for a long period or perhaps permanently asporogenous. The asporogenous strains differ from the original sporogenous strain by a decreased virulence for the mouse, an increased sensitivity for the bacteriophage and loss of some biochemical properties.

K. T. Wieringa, *Bacillus agar-exedens*, a new species, decomposing agar. Antonie van Leeuwenhoek **7**, 121, 1941.

In stable manure, leafmold and soil (especially those with a high content of organic matter) agar decomposing bacilli are relatively common. The occurrence of different types of these bacteria — *Bacillus agar-exedens* — in materials with a high content of organic matter suggests the presence in these materials of higher organic compounds, especially apt for their nutrition.

K. T. Wieringa, The formation of acetic acid from carbon dioxide and hydrogen by anaerobic spore-forming bacteria. Antonie van Leeuwenhoek **7**, 251, 1941.

Further experiments on an anaerobic bacillus synthesising acetic acid from CO_2 and H_2 are described. The organism in question was classified as *Clostridium aceticum* nov.spec. Acetic acid is also formed from sugar. It was shown that at any moment the number of H_2 molecules used for synthesis is proportional to the number of molecules present. Continuous provision with H_2 and CO_2

influences the rate of the process favourably. In such conditions a culture may absorb as much as 4.5 liter of H_2 a day at 30° C. The pH range of *Cl. aceticum* is between 7.5 and 10.5, the optimum being between 8 and 9.

A growth promoting substance is present in alkaline mud extract. This substance can be concentrated by means of absorption or precipitation. Its nature is still unknown.

A. Tasman en A. C. Brandwijk, Stofwisselingsproeven met *C. diphtheriae*. IV. (Experiments on the metabolism of *C. diphtheriae*. IV). Geneeskundige Gids **19**, 240, 260, 1941.

The production of diphtheria toxine cannot be induced under anaerobic conditions; either by culturing in the usual way or by preventing the formation of a pellicle by shaking the medium. When, however, the organism is cultured in aeration flasks, the dissimilation of the sugar takes a completely normal course. The figures and curves bearing on the fermentation of the sugar supplied, the course of the pH and the production of CO_2, NH_3 and toxine agree completely with those obtained in former experiments with „stagnant" cultures. Diphtheria toxine appears to be able to stand a strong aeration with oxygen. The experiments described confirm the assumption that the toxine is an exotoxine.

A. Tasman et A. B. F. A. Pondman, Sur la fermentation de la glucose par *Cl. tetani*. (On the fermentation of glucose by *Cl. tetani*). Antonie van Leeuwenhoek **7**, 169, 1941.

In contradiction with the investigations of Boorsma, Prévot and Veillon respectively Prévot and Kirchheiner it has not been possible to ferment glucose, either by three strains of *Cl. tetani* or by a bacteriologically pure strain, isolated from the strain „L", which has been made use of by the former investigators. So it has to be admitted along with Kolle and Hetsch and many other investigators that it is absolutely impossible to make *Cl. tetani* ferment glucose. The gaseous products, carbon dioxide and ammonia are exclusively due to the decomposition of nitrogen products, derived from the peptones and amino acids added to the nutrient medium. It is very probable that the curious results presented by Boorsma, Prévot and Veillon are due to the use of a bacteriologically impure culture. The formation of tetanic toxin is certainly favoured by the adding of glucose to the tetanic broth. The cause of this favourable action is still unexplained.

A. R. Prévot et H. J. Boorsma, Au sujet de la fermentation du glucose par *Pl. tetani*. (On the subject of the fermentation of glucose by *Pl. tetani*). Antonie van Leeuwenhoek **7**, 239, 1941.

The impurity of the strain used is disclaimed.

A. TASMAN et A. B. F. A. PONDMAN, Sur la fermentation de la glucose par *Cl. tetani*. (On the fermentation of glucose by *Cl. tetani*). Antonie van Leeuwenhoek **7**, 242, 1941.

The authors keep to their opinion as to the impurity of the strain such as it arrived in their possession.

E. C. WASSINK and A. MANTEN, Some observations on the utilization of organic compounds by purple sulphur bacteria. Antonie van Leeuwenhoek **8**, 155, 1942.

Enrichment cultures of purple sulphur bacteria from Delft mud were made in inorganic media. By means of shake-cultures in agar with inorganic salts it was attempted to isolate a strain, the growth of which was favoured by organic substances. This aim could not be attained as all strains isolated throve better in media containing sodium malate than in completely inorganic ones. So it may be concluded that purple sulphur bacteria, at least those isolated from ordinary mud, show a better development under photo-heterotrophic than under photo-autotrophic conditions.

A. MANTEN, The isolation of *Chromatium Okenii* and its behaviour in different media. Antonie van Leeuwenhoek **8**, 164, 1942.

An enrichment culture of *Chromatium Okenii*, obtained from mud of a ditch in Delft, was used for isolation of this species. The bacteria of the strain, isolated by making 5 successive shake cultures in an agar medium were, however, less than half the size of *Ch. Okenii* when cultivated under the same conditions as the bacteria in the original culture. By variation of the culture conditions for the pure strain, it appeared that in a mineral medium containing fairly high concentrations of sodium thiosulphate and fairly low concentrations or sodium malate, bacteria developed of nearly the same size as present in the enrichment culture. This supports the view of WINOGRADSKY that media of this type offer the most natural conditions to purple sulphur bacteria.

MARIE P. LÖHNIS, Sind hautbildende Hefen befähigt elementaren Stickstoff zu assimilieren? (Are pellicle forming yeasts able to assimilate elementary nitrogen?) Antonie van Leeuwenhoek **9**, 133, 1943.

It was tried whether the results reported by FREI as to the assimilation of atmospheric nitrogen by *Pichia membranaefaciens* and *Mycoderma vini* could be reproduced. Although the same experimental methods were applied, no assimilation of nitrogen could be ascertained. This difference in results is left unexplained.

K. C. WINKLER, Iets over de stikstof-stofwisseling bij *Bact. coli* (Aspects of the nitrogen metabolism of *B. coli*). Chem. Weekblad **40**, 147, 1943.

The quotient of the decrease of ammonium in the medium and the number of bacteria present is determined after various intervals in a medium containing ammonium sulphate as the sole source of nitrogen on which *B. coli* was cultivated. Along with the increase in age of the cultures the quotient decreases considerably (1000 × or more).

As it is impossible that the bacteria would have contained initially a 1000 fold amount of nitrogen, it is to be accepted that at the start a part of the ammonium is changed into amino-nitrogen which is not taken up by the bacteria. This may lead to the supposition that the synthesis of amino acids is an exogenic process located in the membrane of bacteria. Amino acids are probably produced already in the lag phase; perhaps a definite external concentration has to be reached before the synthesis of protein may occur.

J. D. TAK, On bacteria decomposing cholesterol. Antonie van Leeuwenhoek **8**, 32, 1942.

It has been definitely proved that in suitable enrichment media in which cholesterol is the only source of carbon this compound disappears with relatively great speed. Continuous shaking of these cultures greatly favours their development. From the enrichment cultures three *Mycobacterium* species have been isolated which proved to be able to attack this compound also in pure culture. One of these species which possesses this property in a very marked degree does not seem to have been described earlier. For this species the name of *Mycobacterium cholesterolicum* has been proposed. Strains of *Mycobacterium phlei*, *M. lacticola*, *M. berolinense*, *M. salmonicolor* and of *M. rubrum*, taken from the collection of the Laboratory of Microbiology at Delft, were also able to decompose cholesterol.

A. J. KLUYVER and A. MANTEN, Some observations on the metabolism of bacteria oxidizing molecular hydrogen. Antonei van Leeuwenhoek **8**, 71, 1942.

Enrichment cultures for hydrogen oxidizing bacteria enabled the authors to isolate four different species having this faculty, one of which apparently was identical with *Hydrogenomonas flava* Niklewski. This strain proved to be suitable for manometric experiments with resting bacteria. With suspensions of these bacteria manometric experiments regarding the gaseous metabolism have been performed with the ultimate aim of contributing to the solution of the problem of chemo-autotrophic carbon dioxide

assimilation. It was found that the bacteria only brought about hydrogen oxidation, if they had also been grown under autotrophic conditions; heterotrophically grown bacteria did not oxidize molecular hydrogen, although they were able to respire normally with organic substrates. In contrast herewith the autotrophically grown bacteria proved to be able to bring about both types of oxidation. Moreover, on addition of both hydrogen and lactate these substances proved to be oxidized simultaneously, the rate of each of these oxidations being unimpaired. Under certain conditions the total gas consumption was even unmistakably enhanced. These results seem to warrant the conclusion that hydrogen oxidation asks for a special catalytic system independent of the catalysts active in the normal respiration process. Apparently the special system is only built up when the bacteria are grown under autotrophic conditions and the faculty to synthesize this system is gradually — and after some time irreparably — lost on cultivation under heterotrophic conditions.

Finally it has been shown that bacteria, which were active as far as hydrogen oxidation was concerned, were completely unable to bring about a reaction between hydrogen and carbon dioxide. Herewith the theory regarding the nature of the metabolism of hydrogen oxidizing bacteria already advanced in 1906 by KASERER and still recently defended by BURK has been definitely refuted.

S. J. BUREMA and K. T. WIERINGA, Molydenum as a growth factor of *Azotobacter chroococcum*. Antonie van Leeuwenhoek **8**, 123, 1942.

Though the discovery of molybdenum as an element necessary for the growth of *Azotobacter* enables us to cultivate this organism in a purely synthetic medium, free of combined nitrogen, the optimal medium for its development is not known as yet. Evidence is given that organic matter of the soil is highly favourable. Molybdenum acts as a reductor in the assimilation of atmospheric nitrogen. For the reduction of nitrate nitrogen less Mo is needed than for the reduction of free nitrogen.

A. QUISPEL, The lichenisation of aerophilic algae. Proc. Ned. Akad. van Wet. **45**, 276, 1942.

The fungal symbionts in lichenized algal covers can be cultivated with more success than true lichen fungi. Their great similarity to the latter makes it probable that they are related to certain true lichen fungi and that this alga-fungus symbiosis is comparable to the lichen symbiosis. In consequence they form an excellent object for the study of this symbiosis. The fungi are unable to fix atmospheric nitrogen. They cannot develop without aneurin, which they

obtain in nature from their algal partner. In none of the cultures on various media, the presence of lichenic acids or similar products could be detected. On the contrary it appeared that the alga *Apatococcus* is the producer of a remarkable metabolic product, called apatococcin, with the tentative formula $C_{23}H_{35}O_4N$. Some chemical properties of this substance are described. A relationship with certain lichenic acids is suggested.

A. Quispel, The mutual relations between algae and fungi in lichens. Diss. Groningen, 1943. Recueil des Trav. bot. Néerl. **40, 413, 1943.**

The lichen-symbiosis was investigated by means of experiments with pure cultures of the components. As lichen-algae some *Cystococcus* species were isolated, the only lichen-fungus investigated was *Xanthoriomyces parietinae*. As an orientation, however, a great many experiments were performed with the fungi which are living in symbiosis with the aerial algae *Pleurococcus* and *Apatoccus*, as it appeared that these fungi are closely related to true lichen-fungi, whilst their growth-velocity is much better. In consequence they are an excellent object for the study of lichen-symbiosis. As far as possible the results obtained with the investigation of these fungi were tested upon *Xanthoriomyces*.

It appeared that the fungi did not develop in synthetic culture solutions without the addition of certain nutrilites (aneurin, β-alanin and other bios substances). The lichen-algae can provide the fungi with these nutrilites. These algae themselves were stimulated by the addition of asparagin, nicotinic acid and certain bios substances, when developing in organic culture solutions. In inorganic solutions a good development could only be obtained after the addition of a small amount of ascorbic acid. It is very probable that the lichen-fungi are able to stimilate the photosynthesis of the algae by the production of ascorbic acid or a related substance.

The fungi did not produce lichenic acids in cultures. On the other hand the alga *Apatococcus minor* synthesizes a remarkable metabolic product, called apatococcin, which most probably is related with certain aliphatic lichenic acids.

An investigation of the water-household of some lichens showed that the protective influence of the fungus against a desiccation of the algae is merely very small and can be only perceived when the desiccation is not too intense. The final conclusion is that the lichen-symbiosis may be regarded as a „mutualistic symbiosis" in which the exchange of nutrilites plays an important role.

H. Bouwens, Investigation of the symbiont of *Alnus glutinosa*, *Alnus incana* and *Hippophae rhamnoides*. Antonie van Leeuwenhoek **9**, 107, 1943.

The cultivation of the symbiont from the various nodules was tried on many culture media. These experiments were carried out in all months of the year. Next to *Bacillus subtilis* and a few bacteria and moulds in merely few cases *Actinomyces* developed. Any *Actinomycetae* occurring were kept in cultivation and tested as inoculum. Never could any development of nodules be induced by them in synthetic pure cultures 350 of which were tested. Inoculation with the strain *Actinomyces alni* of von Plotho gave negative results. In sterilized soil in open pots 2 out of 100 uninoculated plants bore nodules, so the rare positive results among the inoculated plants are deemed valueless. It is claimed that the results of von Plotho arrived at by the same means and on fewer plants do neither admit any valid conclusion as to the identification of the endophyte of *Alnus*.

Marie P. Löhnis, De symbiose van *Bacterium radicicola* met vlinderbloemigen. (The symbiosis of *Bacterium radicicola* with leguminosae). Vakblad voor Biologen **25**, 27, 1944.

The investigations on the symbiosis of nodule organisms and their hosts, carried out after the publication of the monograph of Fred, Baldwin and McCoy in 1932 are reviewed. Stress is lain on the part of the host in the symbiotic fixation of nitrogen.

P. B. Rottier, Fluorometrische en spectrophotometrische bepaling van lactoflavine in micro-organismen. (Fluorometrical and spectrophotometrical estimation of riboflavin in micro-organisms). Thesis, Delft, 1942.

Riboflavin is estimated nearly exclusively by means of three methods, *viz.*, biologically, spectrophotometrically and fluorometrically. As the author is of the opinion that it has yet to be established that the biological method will cover the total amount of riboflavin, he has — in collaboration with other members of the Biophysical Research Group in Utrecht — merely studied the physico-chemical methods.

By means of measuring the absorption spectra it has been established that a sufficient purification of the extract could not be realised without considerable losses of riboflavin. Thus the spectrophotometrical method is inexact. Merely by applying an extrapolation of the complete absorption spectra of the non-purified extracts in order to determine the absorption of the light by these impurities, it has been possible to arrive at approximately correct results.

More exact results may be attained by the fluorometric method.

In order to arrive at this aim it has previously to be made sure of that exclusively the fluorescence of riboflavin is measured. A special screen for the elimination of fluorescence of any impurities present has to be applied. Further the decreasing influence on the fluorescence caused by the absorption of the incident light in the extracts and by the presence of substances which will extinguish the fluorescence has to be calculated.

A calibration diagram has been designed which permits the exact reading of the amount of any fluorescent substance in an extract by means of the estimation of the intensity of fluorescence of two dilutions of this extract.

The extraction of the riboflavin from micro-organisms appeared to be quantitative merely when carried out by means of heating in sulfuric acid of at least 3 %.

Some estimations have shown that micro-organisms are richer in riboflavin than had been believed previously.

G. J. M. VAN DER KERK, Onderzoekingen over de bioluminescentie der lichtbacteriën. (Investigations of the bioluminescence of the luminous bacteria). Thesis, Utrecht, 1942. Cf. also: A. J. KLUYVER, G. J. M. VAN DER KERK and A. VAN DER BURG, The effect of radiation on light emission by luminous bacteria. Proc. Ned. Akad. v. Wet. **45**, 886, 895, 1942.

The investigations made so far prove that in all probability biolumescence is in all cases due to the oxidation by molecular oxygen of a thermostabile compound of low molecular weight: luciferin, through the intermediary of an enzyme: luciferase. For luciferin it has been made acceptable that its molecular structure includes two oxidation possibilities: the reversible ,,dark"oxidation with oxygen or other inorganic oxidiser to dehydro-luciferin is probably based on the presence of a polyphenol grouping (ANDERSON), whereas in the irreversible ,,luminescent" oxidation with oxygen and luciferase a—$CO.CH_2OH$-group is oxidised to a—COOH-group (CHAKRAVORLY and BALLENTINE). Fresh efforts were made to separate the luminous system from the bacterial cell. Action of liquid air on *Photobacterium phosphoreum* did produce a cell desintegration, but no extra-cellular luminescence resulted. Attempts to extract from strongly luminescent bacteria a substance which would be able to restore the luminous capacity of bacteria darkened by exhaustion also led to negative results.

Arguments had been offered that a flavin-enzyme might cooperate in the light reaction. In order to test the possibility that in bacterial luminescence some special derivative of riboflavin would take part, a flavin in the form of its tetra-acetate has been isolated from *Ph. phosphoreum*. As to its melting point and its absorption spectrum this compound proved to be fully identical with synthetic riboflavin-tetraacetate, a result which is unfavourable for the riboflavin hypothesis. As chemical investigation seemed

to offer little prospect an indirect method for the identification of one of the components of the luminous system was taken up.

It had already been proved by VAN SCHOUWENBURG and VAN DER BURG for luminous bacteria that the intensity of the emitted light is reversibly reduced by irradiation. Apparently a photochemical conversion of one of the components of the light emitting system occurs. This radiation effect proved to be clearly dependent on the wave-length. A study of the radiation effect seemed, therefore, to open the possibility of determining an „inactivation spectrum" which at the same time would be the absorption spectrum of that component of the light emitting system which has absorbed the active radiation.

The question was examined in which way the required absorption coëfficient for a definite wave-length will depend on the experimentally determinable quantities, *viz.*, the intensities Y_0 and Y of the bacterial light before and after irradiation. By calculating the photochemical action per incident quantum, a quantity was obtained, defined as „specific photochemical effect" which forms a direct measure for the required absorption coëfficient. The curve representing the relation between the wave-length and the corresponding specific photochemical effect — the inactivation spectrum — will represent at the same time the absorption curve in a relative measure of the compound which has absorbed the photochemically active radiation.

Homogenously grown plate cultures were used, which were irradiated with the light of a mercury lamp. The intensity of the bacterial light before and after irradiation could be estimated for a great number of wave-lengths by means of the photographic method.

The photochemical inactivation spectrum of *Ph. phosphoreum* having been estimated, within wide limits the photochemical action proved to be satisfactorily proportional to the time of irradiation and to the irradiation intensity, so that the Bunsen-Roscoe law appears to hold for the photochemical conversion under examination. The inactivation spectrum shows marked maxima at 405 to 410 mμ and 290 mμ, a minimum at about 350 mμ and further indications for two additional maxima at about 430 and 320 mμ.

The recovery of the light intensity after irradiation was studied. It appeared that those parts of the culture which were irradiated with wavelengths from the region < 300 mμ showed a secondary effect: after an initial increase in light intensity to a value exceeding that of the adjacent non-irradiated parts of the culture, a complete extinction occurs, owing to the death of the bacteria. A quantitative study of the course of their recovery process showed that up to 30 minutes after the end of the irradiation this course is the same for the entire wave-length region examined, and that only after this period the above mentioned secondary effect becomes manifest. From this it was inferred that the decrease in light

intensity owing to irradiation is primarily due to the photochemical conversion of one and the same compound, and that consequently the inactivation spectrum actually represents the absorption spectrum of one single compound.

It has been established that the presence of oxygen is essential for the occurrence of the radiation effect on the light emission of *Ph. phosphoreum*.

It is discussed whether a photo-sensitizer could have been responsible for the photo-inactivation observed. The action of riboflavin or a carotene as such could be conclusively refuted; and it was considered less probable that a so far unknown sensitizer would be involved. Various considerations led to the view that with great probability dehydro-luciferin has to be considered to be the photosensitive component. Oxygen will be essential for the photo-inactivation as only then a larger quantity of dehydro-luciferin is present in the cell.

Finally the author tried to answer the question whether the absorption spectrum determined might contribute to the development of a structural formula of dehydro-luciferin. Arguments could be given in favour of the view that dehydro-luciferin might be a derivative of 1,4-naphthoquinone, in which the $CO\text{-}CH_2OH$-group is a direct substitute of the quinone nucleus. For luciferin a corresponding naphthohydroquinone structure was deemed the most probable.

C. J. P. Spruit and A. L. Schuiling, On the influence of naphthoquinones on the respiration and light emission of *Photobacterium phosphoreum*. Recueil des travaux chimiques des Pays-Bas **64**, 219, 1945.

By addition of naphthoquinone and some of its derivatives in low concentrations to a suspension of *Photobacterium phosphoreum*, both the light emission and the respiration of the bacterial suspension are inhibited, the light emission much more strongly than the respiration. This inhibition is brought about by the naphthoquinones acting as hydrogen carriers, thus shifting the luciferin system in the bacteria into a more oxidised state. The hydrogenation of the naphthoquinones was shown to be reversible and the naphthoquinones are dehydrogenated by a KCN sensitive catalyst. This result leads to a modification of van Schouwenburg's scheme of respiration in luminous bacteria. Bacterial luciferin has a normal redox potential (E'_0; pH = 7) of the order of —50 mV. The inhibition is not determined by the concentration of naphthoquinone in the medium in which the bacteria are suspended, but by the ratio of the number of bacteria to the number of molecules of naphthoquinone added, indicating that this compound is practically completely absorbed by the cells.

E. C. Wassink, On the ratio between the uptake of carbon dioxide and of the hydrogen donor in purple sulphur bacteria. Enzymologia **10**, 257, 1942.

In suspensions of the purple sulphur bacterium, *Chromatium*, strain D, in phosphate buffer, pH 6.3, temperature 29° C., the ratio between the amount of carbon dioxide, and the amounts of various hydrogen donors taken up in photosynthesis, was determined manometrically.

The ratio thiosulphate: CO_2 was measured by supplying limited amounts of sodium thiosulphate from a side bulb, and measuring subsequently the corresponding total amount of CO_2 assimilated. Blanks receiving no dosage were run parallelly in each experiment. In these the gas uptake always was very low. The ratio thiosulphate: CO_2 was 3.75, in good agreement with the value previously found by Eymers and Wassink following another method.

In all cases studied the ratio's were practically independent of the magnitude of the amounts supplied, thus the first step of the conversion seems to run to completion before subsequent conversions play an appreciable rôle.

The rate of photosynthesis was always directly proportional to the concentration of the supplied compounds as far as this concentration was low.

E. Katz, E. C. Wassink and R. Dorrestein, On some methodical problems in the study of photosynthesis of unicellular organisms. Enzymologia **10**, 269, 1942.

Chromatium, strain D, again was used. The influence of bacterial concentration in manometric measurements of photosynthesis was measured and discussed. Both gas exchange and fluorescence were considered. In the case of gas exchange the light gradient in the suspension is important for the shape of the curve showing rate of photosynthesis versus incident light intensity. In concentrated suspensions the average light intensity is lower and the gradient larger. A photosynthesis irradiation curve for the average cell may be derived which shows a rather sharp transition between light limitation and light saturation. The possible reasons causing a transition range are discussed. Concerning fluorescence the absorption of fluorescence light within the suspension is reponsible for the observed fact that the fluorescence transition point intensity depends less upon concentration than that of gas exchange.

In direct and alternating current sodium light no differences of the curves representing the rate of photosynthesis against light intensity were found.

Corrections for small differences in the bottom area of the vessels used in the same series are discussed; the corrections to be applied depend on the bacterial concentrations used.

E. C. Wassink, E. Katz and R. Dorrestein, On photosynthesis and fluorescence of bacterio-chlorophyll in *Thiorhodaceae*. Enzymologia **10**, 285, 1942.

Parallel measurements of gas exchange in photosynthesis, and of fluorescence of bacterio-chlorophyll were performed with suspensions of purple sulphur bacteria, *Chromatium*, strain D, under a large variety of external conditions in order to investigate to which extent bacterio-chlorophyll is involved in the chain of photosynthetic reactions.

The gas exchange shows the same relation to the incident intensity of light as in green plant cells, indicating that also in the bacteria the process of photosynthesis consists of light-sensitive and light-insensitive links.

The curves of fluorescence of bacterio-chlorophyll against the incident of intensity of light are bent curves, consisting of an „initial" slope (at low incident intensities), a transition range, and a much steeper „final" slope (at high intensities). This indicates that the acceptor of the excitation energy is present only in a limited amount. In a suspension to which no hydrogen donor was added the transition between the initial and the final slope is found at a low incident intensity, upon supply of hydrogen donor it shifts towards a higher intensity, so that the fluorescence-yield at high light intensities is decreased. It is of special importance that this decrease of fluorescence-yield is also observed if only a small rate of photosynthesis is allowed, *e.g.*, in the absence of carbon dioxide. It is thus concluded that the hydrogen donor is primarily connected with the transfer of light energy from bacterio-chlorophyll to the energy acceptor. Above a certain concentration of hydrogen donor fluorescence is not influenced further; at about the same concentration also the rate of photosynthesis reaches its maximum. This leads to the conclusion that in the presence of an excess of hydrogen donor a certain system becomes the limiting factor both for energy transfer and for photosynthesis. With excess of hydrogen donor the energy transfer (studied by observing fluorescence) and the rate of photosynthesis depend on temperature in the same way. This indicates that the limiting system mentioned is not the system of energy transfer itself, but an enzymatic reaction in which the hydrogen donor is involved. This implies, furthermore, that the hydrogen donor does not occupy the transfer system as such but gives rise to the formation of the energy acceptor by the mentioned reaction. This reaction was found to be markedly sensitive to pH, which sensitivity was different for various hydrogen donors. In experiments in which hydrogen and thiosulphate were simultaneously supplied, the parts of the mentioned enzymatic system reacting with either of these donors could be determined. So far these determinations were only carried out at 29° C., pH 6.3. Then about 68 % of the system was engaged in the reaction with thiosulphate, and thus 32 % in the reaction with hydrogen. The uptake of carbon dioxide against incident intensity shows an S-shape. Some evidence

is presented in favour of the view that the „loss" at low intensities is only apparent, organic hydrogen acceptors which do not reflect in the manometric measurement, playing then a relatively more important rôle than they do at higher light intensities.

Carbon dioxide does not appear to interfere with the transfer of energy since fluorescence is not fundamentally influenced by withdrawal of CO_2. Neither have cyanide and hydroxyl amine a significant influence upon fluorescence. It is concluded that, besides the system reacting with the hydrogen donor, a second dark enzyme-system exists, at which carbon dioxide is converted, and which is sensitive to cyanide and to hydroxyl amine.

Sodium azide and ethyl urethane also affect the latter system and moreover compete with the normal energy acceptor at the system of energy transfer.

It follows from these observations that the „BLACKMAN"-characteristics of the gas exchange-irradiation curves can be due to two different processes, *viz.*, 1) a process in which the hydrogen donor is converted into a substance involved in the transfer of energy; it is sensitive to temperature and pH, insensitive to cyanide, and precedes the transfer of light energy; 2) a process in which carbon dioxide is involved, and obviously reduced; it is sensitive to cyanide and hydroxyl amine, and presumably also to temperature, and it follows the transfer of light energy.

The experimental results are in accordance with the assumption that the latter system is a dehydrogenase, the substrates of which are the hydrogen donors. It is assumed that hydrogen is transferred from these substances to a substance or to substances present at the transfer system, which thus become active as energy acceptors.

R. DORRESTEIN, E. C. WASSINK and E. KATZ, Theoretical considerations concerning the relation between photosynthesis and fluorescence of bacterio-chlorophyll in purple sulphur bacteria, with an outlook on the comparative physiology of photosynthesis. Enzymologia **10**, 355, 1942.

It was attempted to construe a connection between the experimental results described in the preceding paper and the results obtained earlier by ORNSTEIN, WASSINK, and collaborators for *Chlorella*. The scheme of photosynthesis to be developed for *Chromatium* can be denoted as an extension of the one proposed for *Chlorella*. The transfer of energy is preceded by a dark process in which the hydrogen donors react, and followed by a dark process in which CO_2 or a derivative of it, is reduced.

A mathematical description of the picture has been given, which also can be considered as an extension of the formulation given for *Chlorella*-photosynthesis by ORNSTEIN et al. Finally a general survey of data in connection with a discussion of recent literature and an outlook on the comparative physiology of photosynthesis has been presented.

E. C. Wassink et J. A.H. Kersten, Observations sur la photosynthèse et la fluorescence chlorophyllienne des diatomées. Enzymologia **11**, 282, 1943—1945.

Photosynthesis was studied with diatom-material collected in nature. It consisted nearly wholly out of *Nitzschia dissipata* (Kütz) Grun.; after purification by centrifugation it could be preserved in an active state in the refrigerator for several weeks. Additional observations were made with uni-algal cultures obtained from this material; the species isolated was determined as *Nitzschia spec.* **cf.** *ovalis*, and represents probably a culture form of the above mentioned one. Photosynthesis was measured with the Warburg-technique in Warburg-buffer No. 9 or in a modified Richter-solution in contact with air containing 5 % of CO_2. Fluorescence was measured with a photocell-amplifier-apparatus. Sodium light or yellow light, isolated by filters from an incandescent lamp, were used as light sources. Photosynthesis showed much the same characters as in *Chlorella*, remarkable were the large capacity of the Blackman-system, *e.g.*, at 6° C. high light intensities were still efficiently utilisable, and the strong respiratory intensity. Cyanide and ethyl urethane acted much the same as in *Chlorella*. Respiration was also strongly sensitive to temperature. After correction for respiration photosynthesis in its light-limited region showed no significant temperature sensitivity. The quantum efficiency ($O_2/h\nu$) in Warburg buffer was about 1/13 for the cells collected in nature; the fluorescence-yield about 0.25 %; the photosynthetic quotient (O_2 CO_2) about 1.1 equal to the value found for *Chlorella*. Thus, fat formation does not seem to take place directly in photosynthesis, as was also found by Barker. Fluorescence showed a remarkable new phenomenon, *viz.*, a considerable d e c r e a s e in yield beginning at about the light intensities at which photosynthesis is saturated. It was considered of importance that this decrease is not found when photosynthesis is limited by CO_2-supply, and neither during the initial stages of an illumination. From observations with *Chlorella*, Wassink and Katz had previously concluded that in *Chlorella* the energy transfer is impeded by a reduced state of the cell. It was now assumed that in the diatoms the reaching of light saturation under normal conditions is determined by the capacity of the O_2-producing (OH-removing) system. Furthermore it seems likely that in the diatoms with their strong respiration part of the transfer system remains reduced as long as photosynthesis is not light-saturated, so that the energy transfer then cannot reach its maximum value. This only becomes possible as soon as the OH-groups formed can no longer be removed quickly catalytically, and can partly be used for establishing a more oxidized state at the transfer system, enabling a better energy transfer, and, thus, causing a lower yield of fluorescence.

Measuring the accumulated productions of many separate expositions to light during only a few (5 or 10) seconds, the „induction" phenomena of photosynthesis were studied with the Warburg

apparatus, so that a series of light intensities could be applied simultaneously. At low light intensities the rate of photosynthesis was the same as under stationary conditions of illumination. Light saturation, however, was reached at lower light intensities and showed a lower rate of photosynthesis. The light saturation-rate in the induction period was strongly dependent on temperature. The saturation value is higher for illumination periods of 10 sec. than for periods of 5 sec. The various observations show that in dark a cyanide-insensitive-dark system is partly inactivated, and is reactivated during illumination. It lays at hand to assume that in the dark this catalyst is reduced by the respiratory activity, and that it is only active in an oxidized state, as was concluded also for the system of energy transfer.

J. DE TEMPE, Alkaloïdvorming door *Claviceps purpurea* (Fr.) Tul. in saprophytische cultuur. (Formation of alcaloids by *Claviceps purpurea* (Fr.) Tul. in saprophytic culture). Thesis, Amsterdam 1945.

Many hundreds of isolations from Spanish, Dutch, Canadian, Polish and Hungarian ergot were tested. Alcaloid formation in saprophytic culture is in principle possible. The absence of sclerotium formation in cultures does not prohibit alcaloid formation. The percentage of alcaloid forming cultures from Spanish commercial ergot was twice that from the ergot of other origin. The ability to form alcaloids decreased rapidly as the isolations grew older. Attempts to avoid the degeneration by using a number of different media in stock cultures were in vain. In peptone-maltose *Claviceps* demonstrated a scantier growth but more often alcaloid formation than in asparagine-saccharose. This was connected with a stronger deterioration in the latter solution. In asparagine-saccharose with tertiary calcium phosphate alcaloid formation appeared more often than in the same solution with secundary phosphate. As an acid solution is better for the preservation of the in water soluble alcaloids this was probably due to a stronger alcaloid formation in the more alkaline solution.

The interference of the many factors that influence the formation of alcaloids in saprophytical culture (and which not all could be regulated) caused a great irregularity in the results.

The cultures exhibited also great differences in mode of growth, colour, spore formation, formation of pseudosclerotia and other qualities.

When newly isolated strains from ergot of good quality (Spanish) were used, and the chemical assay followed directly on the filtering off of the mould deck, then it was possible to demonstrate a measurable quantity (at least 0.01 mg) of alcaloids in more than half of the cultures. The highest outputs of 50 ml cultures were 1.50 mg alcaloid in the mould, 0.21 mg in water soluble alcaloid in the

mould; and 0.90 mg alcaloid in the filtrate. The conditions of the experiment did not give an explanation for those isolated cases of high output, found in different cultures. These quantities must be compared with those of MARTIN, who regularly obtained 2.45 mg alcaloid in the mycelium from 50 ml nutrition agar, and those of BÉKÉSY, who obtained 7.4 mg in the mycelium from 50 ml agar.

In some experiments a strong influence of different kinds of water, used as solvents for the food substances, on the alcaloid formation was observed. A connection between these results and the composition of the different kinds of water could not be ascertained. In extensive experiments, in which traces of many elements were used, no indication was found to attribute these differences to the presence of micro-elements.

In experiments with crude vegetable extracts some influence was found on the growth of *Claviceps*, but more on the rate of growth than on the final output. No influence on the alcaloid formation was found, not even if extracts or products from rye and other cereals were used. Indole-containing substances — such as tryptophane which is closely related to lysergic acid — has no favourable influence on the formation of alcaloids.

The mould crop was greater in diffuse light than in darkness, but greater in darkness at the optimal temperature of 25° C. than in diffuse light at room temperature.

Cultures from single ascospores or conidiospores, or combined cultures, did not form alcaloids with more ease than the cultures of isolations from fragments of sclerotia, that were commonly used. They also showed no other differences. This, and the results of infection experiments on rye makes it probable, that *Claviceps* is homothallic. Polyploidisation with camphor or acenaphthene is perhaps possible. The clones with considerably increased length of the conidia demonstrated usually a decrease until normal dimensions were reached. They were not better suited for alcaloid formation.

Experiments were made to disinfect chemically, according to SCHWEIZER, culture media made of rye. By a thorough disinfection treatment substrates, prepared according to SCHWEIZER, were in some cases free from infections. In extensive experiments with those media prepared from germinating or from ripening rye and disinfected with chemicals, a good development was never obtained.

No factor was found, which forced the moulds to form alcaloids though they were in principle capable of it.

J. MULDER, The development of sulfanilamidopyridine-resistant strains of pneumococci in vivo. Antonie van Leeuwenhoek **6**, 221, 1939—1940.

In mice the pneumococcus strain America type I may become resistant against sulfanilamidopyridine within a few days, in spite of the fact that this strain originally is highly sensitive to the drug.

The young resistant strain is at first less virulent than the sensitive strain, but after several mouse-passages it can easily regain its full virulence. It is possible to make the strain wholly resistant against sulfanilamidopyridine. The young resistant strain may cause a protracted bacteraemia in mice when the latter are treated with the drug. Later on this phenomenon for the greater part disappears.

In clinical practice it is of great importance to count with the development of drug-resistance, and it will be wise to treat serious pneumococcal infections immediately with substantial doses of sulfanilamidopyridine, probably better still in combination with an anti-serum.

P. Lopez Cardozo, De gevoeligheid van dysenteriebacillen voor sulfanilamideverbindingen in vitro en in de cliniek. (The sensitiveness for sulfamilamide compounds of dysentery bacteria in vitro and clinically). Ned. T. voor Geneeskunde **85**, 417, 1941; Cf. also: Chemotherapie bij bacillaire dysenterie. (Chemotherapy for bacillary dysentery). Thesis, Groningen 1940.

The chemotherapy of *Shigella* infections has been seldom studied either clinically or experimentally. For this reason a few in vitro experiments are communicated so far as they are of clinical importance. Bactericidal effect has been demonstrated for sulphapyridine and sulphamethylthiazol. Sulphanilamide has less bactericidal action and acts mainly as a bacteriostatic. The significance of traces of fresh blood is considered. The value of chemotherapy is pointed out, in which respect the importance of maintaining other therapeutic measures is stressed.

H. W. Julius and K. C. Winkler, On the action of sulfanilamide. II. The action of sulfanilamide on catalase. Antonie van Leeuwenhoek **7**, 25, 1941.

According to Mellon, Locke and Shinn, the bacteriostatic action of sulfanilamide is due to the inactivation of (bacterial) catalase and the resulting accumulation of hydrogen peroxide. The probability of this theory is discussed.

Catalase activity was studied by means of *Photobacterium Fischeri*, as an oxygen indicator. By adding peroxide to the tested cultures of bacteria it has been demonstrated that:

I. *Bacterium coli*, *Photobacterium Fischeri* and *Streptococcus haemolyticus* contain catalase. II. Sulfanilamide does not inactivate the catalase in blood. III. Sulfanilamide does not inactivate bacterial catalase nor does it affect the production of catalase in the growing culture containing the drug. So the conclusion is drawn that the assumption of catalase inactivation to be the essential factor in sulfanilamide action on bacteria will not lead to the solution of the problem.

H. W. Julius and A. Salomon, On the action of sulfanilamide. III. The difference between sulfanilamide and sulfapyridine. Antonie van Leeuwenhoek **7**, 77, 1941.

It has been tried to establish whether the difference in therapeutic effect between sulfanilamide and sulfapyridine is due to the action of a pyridine derivative. Various substances were administered to animals infected with pneumococci along with sulfanilamide. Pyridine itself improved only slightly the action of sulfanilamide. Step by step pyridine derivatives were tried. Toluene-sulfonamino-pyridine, inactive as a therapeutic, greatly enhanced the effect of sulfanilamide. So the action proper to sulfapyridine must be attributed to the fact that another activity, next to sulfanilamide resides in the molecule.

Other drugs may have a similar effect. This was noted for chinine hydrochloride and acetanilide. The original sulfanilamide drug has unmistakable advantages. The possibility of finding drugs that might supplement its action in the cases of infections were it has proved unsatisfactory is of great importance.

H. W. Julius and K. C. Winkler, On the action of sulfanilamide. IV. Is the sulfanilamide molecule altered before action? Antonie van Leeuwenhoek **7**, 153, 1941.

The problem of alteration of the sulfanilamide molecule as the *conditio sine qua non* for its action as a germicide is dealt with experimentally.

1) Some drops of a culture solution containing sulfanilamide and inoculated with cocci are brought in a fresh medium when the decrease in bacterial numbers may be expected. The cocci develop in the same way as if they had remained in their original environment.

2) When an inoculated culture solution is re-inoculated after a lapse of three hours, the freshly introduced cocci pass a same lag phase as the cocci of the initial inoculation.

The sulfanilamide as such is the active molecule.

K. C. Winkler and H. W. Julius, On the action of sulfanilamide. V. The action on anaerobic growth. Antonie van Leeuwenhoek **7**, 163, 1941.

The action of sulfanilamide on *B. coli* is not influenced by different oxygen pressures. Sulfanilamide was shown to act on *B. coli* and *Str. haemolyticus* (strain Aronson) cultured anaerobically: *a*). by addi ion of thioglycolate. *b*). in evaçuated Thunberg tubes. The obtained growth curves with anaerobic cultures show the same characteristics (lag time of action, influence of inoculum, counteraction by para-amino-benzoic acid) of sulfanilamide action

as the aerobic controls. The mechanism of sulfanilamide action must be the same in both cases.

The presented facts are incompatible with the claim of MAIN, SHINN and MELLON as to the anticatalase activity of sulfanilamide, no hydrogen peroxide being formed in anaerobic cultures.

K. C. WINKLER, On the action of sulfanilamide. VI. The action on bacterial respiration. Antonie van Leeuwenhoek **8**, 10, 1942.

The oxygen consumption of resting *B. coli* or *Streptococcus haemolyticus* (strain ARONSON) was not influenced by addition of sulfanilamide. When both organisms were cultivated in media containing sulfanilamide and suspended in sulfanilamide containing buffers the oxygen consumption was the same as in control suspensions of normal cultures. The same conclusions held true with regard to dehydrogenation velocity for various hydrogen donators. The inference is that sulfanilamide does not interfere with bacterial respiration. The cause of sulfanilamide action must be looked for in some other process of bacterial metabolism.

K. C. WINKLER and H. W. JULIUS, On the action of sulfanilamide. VII. A sulfanilamide activating substance in horse blood. Antonie van Leeuwenhoek **8**, 86, 1942

A sulfanilamide activating principle was found to be present in red cells of the horse. This activator substance is active in the rather high dilution of 0.5 % haemolysed red cells. The substance or substances are present in the red cells, not in their cell membranes. They seem to be of a protein nature or adsorbed on the protein (haemoglobin). In some media no sulfanilamide action is obtained without the activator. In other media sulfanilamide action, though clearly present, is markedly enhanced. So it must be emphasized, that the substance under discussion is an activator and not a „conditio sine qua non" for the sulfanilamide action and its characteristics. The substance is activating sulfanilamide against streptococci, staphylococci and *B. coli*. The substance is not present in human blood or in the red cells of sheep, rabbits or mice.

H. W. JULIUS and K. C. WINKLER, On the action of sulfanilamide. VIII. The mechanism of its action. Antonie van Leeuwenhoek **8**, 139, 1942.

The theory of KUHN and others, who explain the action of sulfanilamide and its antagonist p. aminobenzoic acid, by supposing the latter substance to be the prosthetic grouping of a bacterial enzyme, is discussed. Various facts which are not in accordance with this hypothesis are considered. It is emphasized that the

growth promoting property and the sulfanilamide antagonizing property of p. aminobenzoic acid need not necessarily be correlated. If p. aminobenzoic acid counteracts sulfanilamide this does not necessarily mean that the sulfanilamide acts by counteracting p. aminobenzoic acid, as for many kinds of bacteria it is by no means proven that p. aminobenzoic acid is a growth factor.

Experiments show that: 1). *B. coli* grows in synthetic media without p. aminobenzoic acid. Still sulfanilamide inhibits growth in this medium and p. aminobenzoic acid is antagonistic. 2). Cysteine is antagonistic to sulfanilamide with streptococci. 3). Peptone is strongly antagonistic for sulfanilamide. This action is not due to p. aminobenzoic acid. 4). p. Aminobenzoic acid is strongly adsorbed by *B. coli*. This adsorption cannot be due to the apoferment. Sulfanilamide is either not adsorbed or if it is, adsorption is within the experimental error.

It is still unknown which part of the bacterial metabolism sulfanilamide interferes with, by supplanting p. aminobenzoic acid or not. Various considerations lead to the assumption that sulfanilamide interferes with protein metabolism. Within this scope some alternative hypotheses about sulfanilamide action are offered.

K. C. Winkler, On the action of sulfanilamide. IX. The action on the synthesis of amino acids by bacteria. Antonie van Leeuwenhoek **9**, 115, 1943.

Studying the action of sulfanilamide on bacterial nitrogen metabolism, it was shown that: *a*). Sulfanilamide does not alter the rate of gelatin-hydrolysis by papain or by the proteinase of *B. pyocyaneum* and *B. prodigiosum*. *b*). Sulfanilamide does not influence the synthesis of aspartic acid from fumaric acid and ammonium chloride by resting *B. coli*. *c*). Addition of single amino acids does not counteract sulfanilamide. *d*). Addition of single amino acids merely accelerates growth slightly; a marked acceleration was obtained only by adding various amino acids simultaneously. *e*). The addition of such an „optimal" mixture of amino acids did not exert any influence on the action of sulfanilamide on growth. As the growth acceleration shows that the bacteria are saved an important output of energy in synthesis as a result of the supply of the amino acids, the author concludes that sulfanilamide action cannot be due to interference with the synthesis of amino acids from inorganic nitrogen.

Considering these facts, the author expects sulfanilamide to perform its action on bacterial growth by interfering with protein anabolism, anywhere in the synthesis of protein from amino acids.

H. W. Julius and K. C. Winkler, On the action of sulfanilamide. X. The mechanism of action of sulfanilamide derivatives in vitro. Antonie van Leeuwenhoek **10**, 1, 1944—1945.

The better activity (in vitro) of various sulfanilamide compounds as compared with sulfanilamide itself is only quantitative, *i.e.*, an equal activity is obtained with lower concentrations. It is shown that the activity of the drugs studied is so narrowly related to their absorption in the bacteria (*B. coli*), that probably the varying activity of the compounds studied is due to differences in adsorbability. For different drugs the absorbed amount was equal for concentrations with equal activity.

The concentration of p. aminobenzoic acid which re-establishes growth — in cultures containing the compounds studied in concentrations of equal activity — was equal in all cases. This fact corroborates the hypothesis, that activity and absorption are correlated and shows that the mechanism of action (in vitro) is the same in all cases.

J. D. Verlinde en J. Zeldenrust, Over het voorkomen van necrose en de oorzaak van de dood bij pneumonieën, welke met sulfapyridine zijn behandeld. (On the occurrence of necrosis and the cause of death in pneumonia which has been treated with sulfapyridine). Ned. T. voor Geneeskunde **86**, 2520, 1942.

The occurrence of necrosis of inflamed lung tissue in 4 cases of fatal pneumonia, which has been treated with sulfapyridine is described. The possibility is considered that this necrosis might be a result of local anaphylaxia and that the fatal results might be entirely or partly an outcome of general anaphylaxia. Based on experiments taken with rabbits, the assumption is defended that the cause of death by pneumonia of human beings who have been treated with sulfapyridine might be a general, and the necrosis in the lungs a local anaphylactic reaction. In fact rabbits can be sensibilized with filtrates prepared from the infected lungs of rabbits. The reinjection of a large amount of such an extract (5cc) can cause death by shock. Extracts of lungs from rabbits which have been treated with sulfapyridine cause death by shock with even a tenth of this amount. Rabbits which have recovered from pneumonia are also sensibilized. With these animals shock occurs after injecting a large quantity of lung-extract from an uninfected rabbit just as well as after injecting a small amount of lung-extract from one that has been treated with sulfapyridine. A chemospecific anaphylaxis against sulfapyridine could not be shown.

J. D. VERLINDE and J. ZELDENRUST, A phenomenon resembling anaphylactic shock after treatment with sulphapyridine. Antonie van Leeuwenhoek **10, 17, 1944—1945.**

Two patients on which a successful operation of the stomach had been performed developed fever some days after the operation, notwithstanding a prophylactic treatment with sulphapyridine and both of them died rather suddenly respectively 9 and 10 days after the operation. On obduction in both cases hemorrhagic serous-cellular bronchopneumonia were found in the caudal parts of the lungs, all the organs were very hyperaemious and the heart did not show any alteration. To account for the fatal course the possibility of chemospecific anaphylaxis to sulphapyridine has been considered. We succeeded in inducing in guinea-pigs by means of sulphapyridine a shock, which, however, did not result in death. Such a shock could be induced as early as five days after sensibilization. The adding of the filtrate of inflammated lung tissue resulted in a deadly shock. In this connection the surmisal was made that also in the patients a sensibilization by sulphapyridine had occurred and that the pneumonia, which as such could not sufficiently account for the death, has furthered the arising of the shock. The various facts are pointed to which disagree with the identification of the phenomenon observed with an anaphalactc shock. It is mentioned, however, that there is a certain agreement with the phenomenon of SANARELLI-SHARTZMAN and that of GLAUBACH.

A. B. GREVENSTUK, Synthese en chemotherapeutisch onderzoek van sulfanilamido-pyrimidinen. (Synthesis and chemotherapeutical investigation of sulfanilamido-pyrimidines). Thesis, Groningen 1942.

In order to investigate the influence of substitution in the pyrimidine nucleus on the activity of the three isomeric sulfanilamido-pyrimidines (2, 5 and 6), a number of substituted sulfanilamido-pyrimidines were synthetised and tested on chemotherapeutic activity. In order to obtain the substituted aminopyrimidines the following three amino-chloro-methylpyrimidines were prepared: 2-amino-4-methyl-6-chloropyrimidine; 5-amino-2-chloro-4-methyl-pyrimidine; 6-amino-2-chloro-4-methylpyrimidine. Further some aminochloropyrimidines and one amino-chloro-dimethylpyrimidine were made.

An investigation of the therapeutic activity of the sulfanilamido-pyrimidine was made in white mice that had been infected with pneumococci. The mice were injected intraperitoneally in a manner that the inoculum contained about 20000 organisms. Once a day 0.4 ml of a 10 % suspension of the drug in water was administered by a stomach tube. The treatment was continued for five days. The effect of the compound in infected mice was traced by suspending a loopful of blood from the tail twice daily in ascites agar and

counting the resulting pneumococcus colonies. By comparing the course of the septicaemia with that in a mouse treated with dagénan and in one only infected with an equal dosis of pneumococci the activity of the compound could be deduced.

The three isomeric sulfanilamido-4-methylpyrimidines have an equal or larger activity as sulfanilamidopyridine; consequently the position of the sulfanilamide group has no influence in this case. The presence of a methyl group in position 4 of the pyrimidine nucleus proved to increase the activity of the compound; this was especially the case with the 6-sulfanilamidopyrimidines. The introduction of a methyl group in position 5 decreases the activity. The presence of several other groups (methoxy, methylthio, phenylthio, anilino) has the same effect, and increases the toxicity in some cases. The last property depends on the position of the sulfanilamido group.

J. DE JONGE, Heterocyclische derivaten van sulfanilamide en hun chemotherapeutische activiteit. (Heterocyclic derivatives of sulfanilamide and their chemotherapeutical activity). Thesis, Groningen 1942.

A number of compounds, related to sulfanilamide, were synthetised and tested on chemotherapeutic activity. Various sulfanilamidothiazoles, with substituents in the positions 4 or 5 in the thiazole nucleus, and 2-sulfanilamidothiazoline, were prepared. These N'-derivatives of sulfanilamide were obtained by reaction of N-acetylsulfanilyl chloride with the appropriate amines in the presence of anhydrous pyridine. N'-derivatives of sulfanilamide of some substituted selenazoles, oxazoles, isoxazoles and triazoles were prepared by the same method.

The new products were investigated on their chemotherapeutic activity against mouse septicaemia caused by pneumococci.

As is well-known, the substitution of a methyl group in position 4 in 2-sulfanilamidothiazole, has no appreciable effect on the activity. Substitution by the ethyl, tert. butyl or phenyl groups especially increases the toxicity, without annihilating the activity. Substitution of a carboxyl group, either in 4 or 5 position, resulted in loss of activity; 2-sulfanilamido-4-methyl-5-carboxy-thiazole, however, proved to be active. The corresponding 4,5-dimethyl compound is somewhat inferior to 2-sulfanilamidopyridine; an activity equal to that of the latter compound was found for 2-sulfanilamidothiazoline. Though toxic, the sulfanilamide of 2-amino-4-methyl-selenazole is still active; the corresponding phenyl compound did not show activity. A remarkable result is found for the isoxazoles; the 5-sulfanilamido-3-phenylisoxazole proved to be as active as 2-sulfanilamidopyridine, whereas the 3-methyl derivative is only slightly active. The sulfanilamido derivatives of oxazole and triazole are inactive. As for 2-amino-4-methylthiazole-5-sulfamide and its derivatives, they were all found to be inactive. The anilide iso-

meric with 2-sulfanilamidó-4-methylthiazole, proved to be very toxic.

H. Veldstra, Nieuwe vooruitzichten voor de chemotherapie van bacteriële infectieziekten. (New prospects for the chemotherapy of bacterial infectious diseases). Chem. Weekblad **39**, 506, 1942.

The action of sulfanilamide is inhibited by p. aminobenzoic acid, thus by a structurally related substance. The antagonistic factor in yeast is probably as well p. aminobenzoic acid. Mc. Intosh and Whitby were the first to point to the possibility that the action of sulfanilamide might consist in the elimination of some mechanism or enzyme essential for the bacterial metabolism. It had in fact been evidenced that p. aminobenzoic acid is an essential growth factor for many microbes. Substances related to p. aminobenzoic acid may also inhibit the function of sulfanilamide, although in a slighter measure. Sulphopantothenic acid and pantothenic acid act antogonistically as well. Assuming that displacement reactions play a part, it is needed in order to combat a definite microbe to detect its growth factors and to synthesise structurally related substances, which, however, cannot act as such. Probably a special pattern of active points is more important than the structure of the whole of the molecule.

In order to detect any occurrence of antagonistic actions, growth factors for green plants were synthetised by the author, e.g. α-naphthalene sulphonic acid in comparison with α-naphthalene acetic acid. No inhibitory action could be traced. Further investigations were carried out with other compounds of the naphthalene series and with the sulphonic acid derivatives of hetero-auxine and related compounds.

J. J. Duyvené de Wit, A. Jaarsveld, B. C. P. Jansen, A. van Luijk, R. Luyken, H. K. Oosterhuis en J. R. Wybrans, De isoleering van een bactericide en fungicide stof uit een penseelschimmel. (The isolation of a bactericide and fungicide substance from a *Penicillium*). Ned. T. voor Geneeskunde **88**, 718, 1944.

The authors isolated from *Penicillium expansum* Westl. a bactericide and fungicide substance termed by them expansine. Empirical formula: $C_7H_6O_4$. The growth of *Pythium mamillatum* is inhibited in a dilution of 1.000.000; *Staphylococcus aureus* in a dilution of 1 : 100.000; *E. coli* and *V. cholerae* in a dilution of 1 : 20.000. In contradistinction to penicilline expansine is rather toxic: 1 mg dayly is the tolerated dose for an adult rat.

SEROLOGICAL INVESTIGATIONS

J. Reurink, Natuurlijke Agglutininen. (Natural agglutinins). Thesis, Amsterdam 1941.

Two theories exist as to the nature of the natural antibodies. The theory of the physiological ripening maintains that the origin lays in an acquired property which develops gradually postnatally. According to the other theory the natural antibodies develop postnatally as a response to the presence of bacterial antigens produced by saprophytes or commensals. It has been established that the normal hen serum can agglutinate a great number of bacteria of various kinds, whilst in serum of newly hatched chickens no agglutinins whatever occur. The latter develop shortly after the hatching. The mode of flocculation and the behaviour in heating indicate that the natural agglutinins in hens belong to the O-type. By means of absorption experiments it could be established that these agglutinins are very specific. It was possible to cultivate out of the intestins of sound hens a bacterium which agglutinated strongly with normal hen serum. It appeared that this bacterium (*Escherichia anindologenes*) had properties in common with *Shigella sonnei*, which bacterium as well is agglutinated strongly by normal hen serum Although the results of these investigations do not permit the author to reject the ripening theory, he points to the fact that the latter in connection with the bacterial character of the natural antigens (character of the flocculation, thermolability and specificity) does not contradict the theory of the active formation of the natural antigens.

I. J. le Cosquino de Bussy en J. J. van Loghem, Natuurlijke agglutinatie van *Staphylococcus aureus*. (Natural agglutination of *Staphylococcus aureus*). Ned. T. voor Geneeskunde **87, 1164, 1943.**

The occurrence of natural agglutinins in man and in rabbits of various ages was studied, both by direct method and by that of Castellani. The hypothesis is advanced that the antigens which are considered as the physiological growth stimuli responsible for the development of the cellular defence apparatus (Glimstedt), also induce the production of humoral antibodies.

W. A. L. Dekker, C. van der Meer and R. Th. Scholtens, The electrophoretic behaviour of *Bacterium typhosum* in relation to the changes of the antigenic structure observed in the smooth-rough variation. Antonie van Leeuwenhoek **8,** 53, 1942.

Reasons are given for the classification of the Vi antigen among the O-antigens. A description is given of a micro electrophoresis cell with which measurements of the migration velocity of bacteria

can be easily performed. The pH-electrophoresis curves of different strains of *B. typhosum*, determined by the method described, leads to a classification in four different groups corresponding closely to four different antigenic types. Most likely the ø-antigens are amphoteric substances with iso-electric points between pH 2.0 and 2.5 (nucleo-proteins?). After heating up to 100° C. the Vi antigen rest, even at pH<2, possesses a negative charge. In all probability it contains groups strongly acid in character (phosphoric acid, sulphuric acid?). It is supposed that the somatic antigen IX is an amphoteric substance with an iso-electric point above pH 4.5. A provisional diagram of the localisation of the antigenic components on the bacterial surface is given.

J. H. Bekker, The antigenic properties of bacterial spores. Antonie van Leeuwenhoek **10, 67, 1944—1945.**

Bacterial spores are antigenic. Spore antigens differ from the antigens of their bacillary forms. Antigens of the spores of various kinds of spore-forming bacilli also differ mutually.

S. J. C. Dunlop, Is haemoagglutinatie en haemolyse het resultaat van de werking van één enkel antilichaam? (Is hemagglutination and hemolysis the result of the action of one single antibody?) Thesis, Leiden 1941.

The author puts the question, whether hemolysis and hemagglutination are symptoms of the action of one and the same antibody. Rabbit serum immunized against sheep erythrocytes was separated into fractions of proteins by means of various methods (according to Doladilhe and Mazille, Thomsen, Rondoni, Pietttre), and in these fractions the content of hemolysines and hemagglutinins was estimated.

The results of his exeriments lead the author to the conclusion, that hemagglutination and hemolysis do not depend on the same antibody.

S. J. C. Dunlop, On a bacterium that, while retaining its full biochemical and serological properties, develops on the agar plate in two colony forms. Antonie van Leeuwenhoek **7,** 33, 1941; Cf. also: Acta Leidensia **15—16,** 167, 1940—1941.

A bacterium has been described which had the property of continually developing on the agar plate in two colony forms, that had the same biochemical and serological properties and did not show any morphological difference either. The cause of this variety in development is left unexplained.

J. H. Bekker and H. H. Vink, Vi-antigen in *B. coli* and Vi-agglutinin. Antonie van Leeuwenhoek **10, 12, 1944—1945.**

The Vi-agglutinin in normal serum occurs in 4—8 %, the Vi-antigen in *B. coli* out of feces of healthy persons in 7—8 % of the cases. The authors could not, however, prove a connection between these two phenomena. The authors present some reasons why such a connection need not to be considered as absolutely impossible after all.

J. D. Verlinde and A. J. van den Hoven van Genderen, The biological identification of native and of cooked proteins. Antonie van Leeuwenhoek **9,** 32, 1943.

In rabbits specific precipitating sera of high titer (up to 1 : 30.000) can be induced with native as well as with cooked proteins by means of single subcutaneous injections with the protein concerned emulgated in vaseline-lanolin. In the experiments muscle protein of cattle and dog serum and hen albumen have been used. The proteins, either cooked or native, are dried, pulverized and emulgated as such in vaseline-lanolin on a waterbath of 40—56° C., some saline or distilled water added if being needed. The injection follows immediately. The precipitating sera against cooked proteins give a precipitating reaction with the homologous native protein as well as with the cooked protein which is dissolved in NaOH.

Ong Sian Gwan, Sur la production d'anticorps au moyen d'un antigène enrobé dans la lanoline-vaseline. (On the production of antibodies by means of an antigen emulgated in lanolin-vaseline). Antonie van Leeuwenhoek **9,** 1, 1943.

Subcutaneous injection of rabbits with proteins emulgated in a mixture of vaseline and lanolin leads to the formation of a much larger quantity of precipitines than obtained with the usual methods of preparing precipitating serum. By night the formation of precipitines is much stronger than by day.

Ong Sian Gwan, Différentiation des protéines de poumon normal et de poumon intoxiqué par le phosgène au moyen de la réaction anaphylactique. (Differentiation of the proteins of normal lung and the lung poisoned by phosgene by means of the anaphylactic reaction). Proc. Kon. Ned. Akad. van Wet. **45,** 774, 907, 1942

Proteins of the normal lung and those of the lung intoxicated with phosgene of the same kind of animal can be differentiated by means of the anaphylactic reaction. The proteins of the intoxicated lung of different kinds of animals contain an identical grouping, formed under influence of the phosgene.

ONG SIAN GWAN, Serologische verschillen tusschen eiwitten of lipoiden van de normale en door phosgeen vergiftigde long. (Serological differences between the proteins or lipoids of the normal lung and the lung poisoned by phosgene). Versl. Ned. Akad. van Wet. **52**, 40, 1943.

The following results show the observed serological differences between the proteins or the lipoids of the normal lung and the lung poisoned by phosgene:

1. An antiserum against an extract of normal lung gives a positive precipitation reaction with the corresponding extract, while it does not precipitate an extract of a poisoned lung.
2. The complement fixation with the same serum performed with a corresponding extract is much stronger than that with an extract of a poisoned lung.
3. An antiserum against an extract of poisoned lung gives a stronger complement fixation with a corresponding extract than that with an extract of normal lung.
4. An antiserum against lipoids of normal lung gives a stronger complement fixation with the corresponding lipoids than that with the lipoids of poisoned lung.

This result is much clearer when a syphilitic human serum is used.

ONG SIAN GWAN, Over de vorming en de eigenschappen van amboceptoren tegen een longextract. (On the production and properties of amboceptors against a lung extract). Versl. Ned. Akad. v. Wet. **52**, 270, 1943.

1. The organs of a rabbit, inoculated with a normal lung extract contain antibodies. The acquired immunity is general.
2. The complement fixation shows the presence of identical molecular groupings in the lung proteins of different animal species, after the action of phosgene.
3. An antiserum against a lung extract gives a positive BORDET-WASSERMANN reaction and a positive complement fixation with the lipoids of lung extracted by means of acetone, ether, petroleum-ether and alcohol.
4. The simultaneous presence of two antibodies or of two antibodies and two antigens may either intensify or decrease the phenomena due to complement fixation, as shown in the case of a mixture of two antigens.

ONG SIAN GWAN, Over een positieve reactie van BORDET-WASSERMANN, verkregen met een serum tegen longweefsel en een vaccine-immuunserum. (On a positive reaction of BORDET-WASSERMANN, obtained with a serum against lung tissue and a vaccine-immune serum). Onderzoekingen en Mededeelingen uit het Instituut voor Praeventieve Geneeskunde **2**, 1943.

Sera of rabbits which have been immunised with lung tissue of the pig give a positive complement fixation test with a watery

extract of the lung, with pure albumens isolated from the lung and with lipoids extracted from the lung. Moreover, these sera give a positive complement fixation test with the antigen of BORDET-RUELENS, used in the reaction of BORDET-WASSERMANN. The serum of a rabbit, immunised with dermovaccine or neurovaccine gives a positive reaction of BORDET-WASSERMANN too, as well as a positive complement fixation test with albumens and lipoids from the lungs of the pig. It is true that normal sera of rabbits also give a positive reaction with the antigens mentioned, but the values (expressed in complement units, according to BESREDKA) for the sera of the immunised rabbits are considerably higher. The results cannot be explained by the FORSSMAN-antigen as cattle, pigs and rabbits do not possess it. It is assumed that the lipoids of these three species of animals have the same molecular grouping.

W. AEG. TIMMERMAN, Are the toxic properties of a staphylococcal filtrate manifestations of one or more constituents? Antonie van Leeuwenhoek **8, 41, 1942**

The experiments show, that under certain well-defined conditions both the lethal and the necrotic potencies of staphylococcal α-toxin can be destroyed, without much damage to the haemolytic power. After 3 days incubation at 37° C. with 0.25 % of formalin, the lethal activity disappears, whereas the haemolytic potency remains high. The same applies for an incubation period of 4 days at 22° C. with 0.4 % of formalin. Under these conditions it is possible to obtain a modified toxin showing solely haemolytic power. This would apparently not be possible if both the haemolytic and the lethal properties were caused by the same toxic principle. These findings, therefore, strongly support the view, that haemolytic and lethal powers are caused by two different constituents of the toxic filtrate. The same findings were obtained when necrotic and haemolytic activities are compared. The experiments do not permit to answer the question whether lethal and necrotic activities are manifestations of one or of two constituents.

A. PONDMAN, Bloedgroepbepalingen in de praktijk. (Blood group estimation in medical practice). Geneesk. Bladen uit Kliniek en Laboratorium **37,** 291, 1940.

A blood group estimation is usually a very simple procedure, sometimes, however, it is difficult or next to impossible to attain a decision. It is always needed to test the red corpuscles as well as the serum they are derived from. A description is given of the blood group estimation such as it has been indicated by the Rijks-Instituut voor de Volksgezondheid, Utrecht. Difficulties may arise owing to infection, lysis, cold-agglutination, difficult demonstration

of agglutinins, the presence of subgroups. As to the latter case α or β agglutinins may be present, having a limited activity with respect to the red blood corpuscles of group A or B and which can also agglutinate the red corpuscles of the O group. To account for this the assumption is made for each red corpuscle to possess the O antigen, on the surface of which the A, B or A and B antigens occur. So the O antigen is more or less separated from the serum. When the covering is very dense an O agglutinin may be expected, this is the so-called special α or β agglutinin. The special agglutinin is harmless when present in the blood of a donor, in the blood of the receiver, however, it may be harmful. A thorough theoretical knowledge and a large experience should be required in the performer of blood group estimation.

S. J. C. Dunlop, On bacteriophage anti-Flexner. Antonie van Leeuwenhoek **9, 41, 1943.**

The Flexner strain KB of Flu from 1921 has given rise to two strains the Flexner strain 38 which has grown SR and the Flexner strain 39 which has grown R. The Flexner strain 39 is at this moment a spontaneously agglutinating, lyso-sensitive strain, while the Flexner strain 38 apparently has kept up its original properties and is inagglutinable and lyso-resistant. Both strains belong to the type X of Andrewes and Inman. The Flexner strain 38, however, appears to agglutinate very specifically with a X serum. This strain can also be lysed by a special group of Flexner phage and not by others. The Flexner strain 38, however, is probably no variation of the strain KB of Flu. The results of Burnet and McKie as to the correlation between antigenic structure of the Flexner strains and their sensitiveness to some groups of bacteriophage anti-Flexner could be confirmed in a high measure.

J. D. Verlinde, The complement fixation test in vaccinia with antigens from the brain, the testis and the skin. Antonie van Leeuwenhoek **7, 111, 1941.**

Eight antigens prepared from the brain, the testis and the skin of rabbits after the tissues had been inoculated with vaccinia virus, have been compared in the complement fixation test with vaccinia-immune-serum. It was rare that a suitable antigen could be prepared from the brain. Antigens from the testis and the skin were always active, but the activity of the former surpassed that of the latter. The best results were obtained with a saline extract 1 : 100 of freshly removed testis; when frozen this extract may be kept for some weeks. In the sera of vaccinated rabbits and of a monkey complement fixing antibodies could be detected regularly, even three months after vaccination. In sera of men who had been

revaccinated more than three months earlier this was only rarely the case.

J. D. VERLINDE, Manifestation névraxiques et histopathologiques, obtenues chez des lapins inoculés par voie souscutanée avec le neurovaccine et le virus de l'herpès. (Cerebrospinal and histopathological manifestations obtained in rabbits inoculated subcutaneously with the neurovaccine and the virus of herpes). Onderzoekingen en Mededeelingen uit het Instituut voor Praeventieve Geneeskunde **1**, 1943.

Neurovaccine and the virus of herpes cannot be shown in the brain of the rabbit after subcutaneous inoculation. If these viruses have been incorporated in vaseline-lanolin, sesame-oil or glycerol, they can always been shown in the blood and often in the brain after subcutaneous inoculation. The rabbits often die after 8—9 days, sometimes with cerebral symptoms. Histologically an acute meningitis or meningoencephalitis of the same type as the vaccinal or herpetic meningoencephalitis with mononuclear and polynuclear perivascular infiltrations are found.

J. VAN DER HOEDEN, De toepassing van het verschijnsel der phagocytose der Brucellosen. (The practical use of the phenomenon of phagocytosis in brucelloses). Tijdschrift voor Diergeneeskunde **67**, 910, 963, 1940.

By adding liquoid to blood the complement becomes inactive. As, the complement being absent, the normal opsonines are inactive and the specific tropins are not yet inactivated, it might be expected that the phagocytosis reaction, carried out with liquoid blood, would furnish specific results. Such was actually the case in man. By adding liquoid to the blood of cow, horse and goat, however, the tropine reaction appeared to be completely inhibited. In blood of rabbits its action varied in strength. The inactivation of the tropins was not caused by a change of the white blood corpuscles, but by the action of the blood liquid, although it is not connected with inactivation of the complement. The liquoid still acts in a very great dilution (end concentration 1 : 4000 to 8000).

In the tropin reaction in brucellosis it is aimed at to detect the specific phagocytosis stimulating agents, the tropins which are thermostabile in contrast with the non-specific opsonins. In the sera to be tested the tropins are kept on during a very long period. The reaction can thus be carried out at any moment.

For the phagocytosis blood of man or animals (cow, horse, sheep and goat) may be used.

In experiments with goats which had been injected with living or killed off cultures of *Brucella Bang*, the tropins appeared as soon as 5 or 6 days after injection, the agglutinins and amboceptors still 1 to 3 days earlier. The three antibodies remained present during several months.

The investigation of the reaction of the blood of 62 men, 112 cows and 66 horses learned that the tropin reaction possesses next to a great sensitiveness a high specificity. In some cases of brucellosis the agglutination and complement fixation tests were negative or merely slightly positive, whilst these sera gave a strong tropin reaction.

An important diagnostic value to the positive tropin reaction is attached in man and cow. In the horse it is of lower value. The positive results in a number of horses that do not clinically suffer from brucellosis, are probably connected with the frequent occurrence of latent infections in horses. The tropin reaction in the diagnostics of brucellosis is an easily practicable quick method, that can be recommended for practical use.

J. VAN DER HOEDEN, A tropin-reaction for the diagnostic of brucellosis. Antonie van Leeuwenhoek **7**, 211, 1941.

Description of a serum test called „tropin-reaction" for the routine diagnosis of brucellosis in man and animals. It is based upon the heat-stability of specific tropins, in contradistinction to the heat-sensibility of the unspecific opsonins. With due regard to latent infections, especially in the case of highly exposed individuals (occupational infections) and in horses, the tropin-reaction is a simple, specific and extremely sensitive diagnostic method, which may prove a valuable addition to the agglutination- and complement-fixation reactions.

J. H. BEKKER and H. H. VINK, Salmonella antigens in *Bacterium colli* and paragglutination. Anthonie van Leeuwenhoek **8**, 134, 1942. Cf. also: Salmonella-antigenen in colibacillen en paragglutinatie. Ned.-T. voor Geneeskunde **86**, 2629, 1942.

The phenomenon of paragglutination with typhoid serum of *B. coli* from the intestins of healthy persons and of patients suffering from typhoid or paratyphoid fever was observed and studied. Evidence could be obtained that the paragglutination was to be attributed to the presence of a common Salmonella antigen (XXVIII), so that it is wrong to make a distinction between paragglutination and co-agglutination. Salmonella antigens were found in various combinations with *B. coli* and different combinations could also be ascertained for the same person. The attention is drawn to the presence of the Vi-antigen with *B. coli*, also from healthy persons and the possibility of its connection with the Vi-agglutinin in the blood of healthy persons is pointed at.

J. MÜLDER, L. BIJLMER en L. VAN TUINEN, De antigène structuur van den in Februari 1939 geïsoleerden stam van influenzavirus. (The antigenic structure of the strain of influenza virus isolated in February 1939 at Groningen). Ned. T. voor Geneeskunde **85**, 1743, 1941.

The influenza strain isolated in the Groningen epidemic of February 1939 was studied in detail for its antigenic structure. Use was made of ferret immune sera and of the specific strains WS, Talmey, Gatenby and Christie. The strain was found to be closely related with the Christie strain, isolated in England in 1937, less closely with the Talmey and remote from the WS and Gatenby strain. The epidemic from which the strain was isolated has been accurately described as to its morbidity, course of illness and nature and number of secondary bacterial infections.

W. J. BRUINS SLOT, Infectieuse mononucleose. (Infectious mononucleosis). Geneesk. Bladen uit Kliniek en Laboratorium **38**, 79. 1940.

A survey is given of the history of the development of infectious mononucleosis and its relation to the glandular fever of PFEIFFER. The identity of both diseases is doubted. A diagnosis can only be established with certainty by means of morphological and serological blood investigation. There exists a relative and an absolute mononucleosis, the latter amounting to 60—90 % of the white blood corpuscles. Serologically it is important that agglutinins against sheep erythrocytes are formed (reaction of PAUL and BUNNELL) which cannot be absorbed by cells from the kidney of guinea-pigs, but can be absorbed by cells from cow blood and can be distinguished in this manner from FORRSMAN antibodies.

The investigations of the author comprehend twenty cases of infectious mononucleosis, observed in the last seven years. They were scattered cases, slight epidemics not having been observed. The aetiology has not been ascertained as yet. According to NYFELDT and SCHMIDT and NYFELDT *B.* (*Listerella*) *monocytogenes hominis* is isolated from blood respectively spinal fluid of patients with infectious mononucleosis. The serum from some of these patients, however, did not show any increase of agglutinion against *Listerella monocytogenes*. For clinical reasons the author claims infectious mononucleosis to be probably a virus disease.

J. H. BEKKER, De beteekenis van absorptieproeven voor de reactie van PAUL en BUNNELL. (The significance of absorption tests for the reaction of PAUL and BUNNELL). Ned. T. voor Geneeskunde **85**, 2197, 1941.

The author describes a method by means of which the agglutinins against sheep red blood cells found in normal serum, in serum of

patients who have received a therapeutic serum injection, as well as in serum of patients suffering from *Mononucleosis infectiosa*, can be differentiated. The method is based on absorption with kidney cells of the guinea-pig and with ox red blood cells. Kidney cells of the guinea-pig absorb the agglutinins from normal serum and from serum of patients who received a serum injection, and not those of the serum of patients suffering from *Mononucleosis infectiosa*. Ox red blood cells absorb the agglutinins after a serum injection and in *Mononucleosis infectiosa*, but as a rule not the agglutinins against sheep red blood cells found in normal serum.

A. Pondman, On the preparation of vaccine against typhus fever and the experiences gathered therewith. Antonie van Leeuwenhoek **10**, 57, 1944—1945.

Experiences have been gathered in the culturing of Rickettsiae in order to arrive at the preparation of a vaccine against typhus fever. The strains are kept active by passage through guinea-pigs. The susceptibility of these animals may strongly vary and depends on their batch of origin. The fertile hen's egg is used as a growth medium for the Rickettsiae. After initial growth in the egg the organism is further grown on a suspension of the yolk sack in saline. When growth was profuse the production of a toxine might cause difficulty, which however, could mostly be overcome either by dilution of the growth medium or by incubation at low temperature. The vaccine is purified by centrifugating of the yolk sac suspension. Before application it has to pass a control on sterility, on harmlessness and on its immunizing value.

S. L. Brug, Op Rickettsia gelijkende vormsels in de menschelijke long. (On corpuscles resembling Rickettsia in the human lung). Ned. T. voor Geneeskunde **85**, 4636, 1941.

Description of intracellular granules found in sections as well in smears of human lungs. The granules greatly resemble Rickettsia. In 21 lungs collected at random they could be found in every case either in the smears or in the sections or in both. Probably they are normal elements of the lungs. When one is searching for virus in human material they may be mistaken for Rickettsia. Also in spleen and kidney these corpuscles were found.

S. D. Liem and F. H. van Thiel, The complement-fixation test for Chagas' disease employing a dried culture antigen. Acta Leidensia **15—16**, 259, 1940—1941.

A complement-fixation test for Chagas' disease, in which a dried culture antigen of *Trypanosoma cruzi* is used, is described. The antigen is checked with good result by means of sera of rabbits immunized with this parasite and of dogs infected with it.

Sera of 367 persons not suffering from Chagas' disease are tested. 2.7 percent cross reactions occur with Wassermann-positive sera. Struma patients don't lead to a positive reaction.

A positive reaction was shown in 4 out of 6 (66 per cent) ulcus molle patient sera. It has been proved by means of the immunization of a rabbit with Dmelcos vaccine that the positive reaction was not a fortuity.

The antigen is very probably tenable unlimitedly. In practice its use yields advantages over the antigens used up to now.

It deserves recommandation to check the value of the antigen in sera of patients claimed to be suffering from Chagas' disease on account of the establishment of the presence of *Trypanosoma cruzi* and not suffering from an ulcus molle infection. This research must be performed in countries where Chagas' disease is endemic.

MEDICAL BACTERIOLOGY AND SEROLOGY

J. H. Bekker, De beteekenis van de fluorescentie-microscopie bij het onderzoek op tuberkelbacillen in sputum. (The significance of the fluorescence microscopy in the examination of sputum for tubercle bacteria). Ned. T. voor Geneeskunde **85**, 3399, 1941.

The author describes the results of a comparative investigation of 1200 sputa, examined according to the Ziehl-Neelsen method and by means of the fluorescence microscope. 183 sputa appeared to be positive according to the Ziehl-Neelsen stain, as well as on application of the fluorescence microscope. 4 sputa were found to be positive only by means of the Ziehl-Neelsen method; in three cases this result was confirmed by means of animal and culture tests, but as to the 4th sputum, the presence of tubercle bacteria could not be proved, neither culturally, nor by means of animal tests, nor clinically. 13 sputa were ascertained to be positive only on applying the fluorescence microscope and in 7 cases this result could be confirmed by means of culture and animal tests. Out of the remaining 6 sputa 3 came from patients who were certainly not suffering from tuberculosis and as to the other 3, the patients suffered from pulmonary tuberculosis, closed or not. With a view to these details, it may be considered as probable that the fluorescence microscopy is a more sensitive method to prove the presence of tubercle bacteria in sputa, but that on the other hand the reliability of the results decrease.

J. H. Bekker en A. Tasman, Het verband tusschen de zuurvastheid en het fluorescentievermogen van den tuberkelbacil. (The relation between the acid-fastness and the fluorescenting action of the tubercle bacteria). Geneeskundige Gids **19**, 623, 1941.

The conclusion of some authors that the acid-resistance and the

fluorescenting action of the tubercle bacteria are due to different principles seems not to be adequate. After ungreasing with alcohol, ether and chloroform the tubercle bacteria loose their acid-resistance but also their fluorescenting action. Therefore these two phenomena seem to depend on one and the same principle *e.g.* the lipoid cover of the bacillus.

G. P. F. Munnik, Het aantoonen van tuberkelbacillen met het fluorescentiemicroscoop. (The detection of tubercle bacteria with the fluorescence microscope). Tijdschrift voor Diergeneeskunde **69**, 287, 1942.

The paper contains a short description of the various apparatus used in fluorescence microscopy, a list of the staining methods in use and the work carried out in this field. Most of the workers have compared this method with that of Ziehl-Neelsen, and studied in how far a greater number of positive results could be attained, and a shortening of the time needed for the examination.

The advantages of the fluorescence method are: 1. great gain of time. 2. possible gain in positives. 3. simple staining method. 4. the use of a dry system. The disadvantages are: 1. the need of working in a darkened room. 2. the great sensitiveness of the method, so that the greater gain in positives may not always be ascribed to the tubercle bacteria. 3. the preparations stained with auramine lose their fluorescent activity rather quickly.

J. H. Bekker en H. H. Vink, Het onderzoek op tuberkelbacillen in weefselsneden met den fluorescentie-microscoop. (The examination of tubercle bacteria in tissue sections with the fluorescence microscope). Ned. T. voor Geneeskunde **87, 10, 1943.**

Description and discussion of the test for tubercle bacilli in tissue sections by means of the fluorescence microscope. The fluorescence microscopy is to be preferred to examination with the Ziehl-Neelsen stain, because of the simpler staining technic, the stronger contrast, the detecting of more bacteria and the time gained.

D. Mulder, Het kweeken van tuberkelbacillen bij de bestrijding der rundertuberculose. (The culturing of tubercle bacteria in the eradication of tuberculosis among cattle). Thesis, Utrecht 1943.

The purpose of this investigation was to introduce a method for the detection — as complete as possible — of animals with open tuberculosis. For that reason the cultivation of tubercle bacteria on an artificial medium from sputum and from other exudates was studied.

Smears of the sputum were stained after ZIEHL-NEELSEN and examined for acid fast bacteria. If these were not found, the sputum was treated with sulfuric acid and cultivated on the LOEWENSTEIN medium. Every precaution was taken to prevent acid fast saprophytic bacteria to be mistaken for tubercle bacteria.

To prove the efficiency of the cultivation method the detailed results of the examination of 43 cows, one horse and one goat were recorded. All these animals showed a positive tuberculin test, but the repeated bacterioscopical examination of the smears had no results. It was possible to cultivate tubercle bacteria in everyone of them within 20 to 30 days.

The results of the examination of smears has been compared by:
1. the staining method of JÖTTEN-HAARMANN.
2. the fluorescence microscopy.
3. the cultivation method.

With the cultivation method many more cases of open tuberculosis were detected than with either of the bacterioscopical methods. In a few cases the results of the inoculation of sputum in guinea-pigs were compared with those of cultivation. The latter method appeared to be not inferior to the first.

Among 235 cows, all giving a positive tuberculin test and residing on farms, where notwithstanding systematic measures against tuberculosis had been applied, a higher number of positive reactors had been found than the year before, 23 cases of open tuberculosis had been detected, by the cultivation method. Of 150 cows, indicated by clinical examination as highly suspected of severe tuberculosis, only 6 open cases were found by bacterioscopical examination. Among the remaining 144 another 22 cases of open tuberculosis were detected by the cultivation method.

The conclusion of the author is that the cultivation method should be employed obligatorily in the systematic eradication of tuberculosis among cattle.

C. F. VAN OYEN, Een kweekmethode voor tuberkelbacillen toegepast bij de bestrijding van de rundertuberculose. (A cultural method for the tubercle bacilli used in the fight against the bovine tuberculosis). Verslagen Tuberculose Studie-Commissie **20**, 19, 1944.

An effective separation on the cattle-farms of animals infected with tuberculosis, according to the tuberculin reaction, from non-infected animals is very difficult. Notwithstanding the measures taken, new infections continually arise among the full-grown and especially among the young cattle.

Among the means available for the detection of spreaders the culturing of tubercle bacilli on the medium of LÖWENSTEIN is discussed in detail. Apparently the culture method furnishes the means to detect the remaining spreaders of bacilli. The results obtained by means of this culture method are compared with those of the bacterioscopic examination of sputum under the fluorescence

microscope. It appears that with the culture method many more open sufferers are detected.

The results of the culture method and of the animal test are compared. Here the number of open sufferers detected are of the same order. The animal test may be slightly more sensitive. It is pointed out that initially one may have mastered the technique of the culture insufficiently which fact enhances these differences.

This method may be applied successfully on cattle farms where a larger group of reacting animals can be found and where repeatedly new infection is detected among the young cattle.

J. H. Bekker, De beteekenis van de verschillende laboratoriummethoden voor het onderzoek op tuberkelbacillen bij den mensch. (The significance of various laboratory methods for the detection of tubercle bacteria in men.) Verslagen Tuberculose Studie-Commissie **20,** 3, 1944.

The quickest way to detect tubercle bacteria in matter taken from tuberculous patients or from such suspected of being tuberculous, is the direct microscopic preparation, the results of which can be increased by concentrating the matter in some way or other. In a considerable part of the cases, however, this method will leave us in the lurch. A much more sensitive method is to try to cultivate the bacteria out of the matter and better still to demonstrate it with the aid of the guinea-pig. While offering a great measure of sensitiveness and reliability, both methods, however, have the disadvantage of being of long duration to which is added, for the guinea-pig experiment, the intercurrent mortality of the test animals. It stands to reason that the best results are obtained by applying the available methods side by side; the results can thereby supplement each other; this is therefore the method followed in the State Institute for Public Health.

J. P. Bijl en J. D. Verlinde, De beteekenis van de virulentie der tuberkelbacillen. (The significance of the virulence of tubercle bacilli). Verslagen Tuberculose Studie-Commissie **17,** 17, 1942.

In order to ascertain whether the virulence of the tubercle bacilli circulating among the population has decreased in the last 50 years, it has been investigated whether the length of life of the guinea-pigs infected with tubercle bacilli has undergone any change. Reliable data have been collected in literature and treated statistically. As the duration of life of the guinea-pigs which had been injected with a same amount of a same culture varied widely, its average has been determined for every group of animals injected with a same material. Out of these data the average length of life of the group of animals which had been infected in a same year

has been determined. The entire material concerns 382 guinea-pigs divided over 37 groups. Statistically no change in the virulence could be ascertained.

A. Ch. Ruys, Kritische beschouwingen over de bestaande opvattingen over de pathogeniteit van de vogeltuberkelbacil voor den mensch. (A critical treatment of the existing views about the pathogenicity of the avian tubercle bacillus for men). Verslagen Tuberculose Studie-Commissie **15, 43, 1941.**

This review of the literature shows that *Mycobacterium avium* can be recognised as a separate variety and that even its strains of weak virulence may be determined by various methods. Not all reports on avian tubercle bacilli in disease of man are complete enough to judge about their pathogenicity. Clinical features characteristic for this infection are nowhere reported. Neither the reaction on tuberculin, nor the serological examination of the patient can furnish any indication as to the existence of avian infection in men. The hypothesis of the activation of a latent infection with this otherwise non-pathogenic micro-organism under definite conditions might explain some of the rare findings of avian tubercle bacilli in men.

J. van der Hoeden, Tuberculose bij zoogdieren, veroorzaakt door het vogeltype van den tuberkelbacil. (Tuberculosis in mammals, caused by the avian type of the tubercle bacillus). Tijdschrift voor Diergeneeskunde **68, 335, 1941.**

A review of literature learns that cases of tuberculosis caused by the avian type have been sporadically observed in various species of mammals. In swine it has been regularly observed. The author's investigations offer the following results:

	strains examined	human	bovine	avian
Man	914	842	72	0
Cow	137	0	133	4
Sheep	3	0	3	0
Goat	17	0	15	2
Swine	169	0	113	56
Horse	14	0	12	2
Dog	8	3	5	0
Cat	6	0	6	0
Wild animals in zoological gardens	12	0	12	0

Nearly all cows and horses with avian infections had severe forms of tuberculosis (miliary dissemination, diffuse catarrhal lung

tuberculosis, large extension). Contrarily to most of similar cases stated in literature the avian infection in swine had mostly caused no striding processes, although a few cases with more severe extension were met with. Both goats had grave cavernous lung processes, characteristic for tuberculosis in these animals. The results of the investigations substantiate the presumption that the tuberculosis of birds may be a source of danger for our domestic animals and that in the fight against tuberculosis in cattle we have to pay attention not merely to the bacteria of the bovine type but to the avian type as well.

J. VAN DER HOEDEN, De typen van tuberkelbacillen bij zoogdieren in Nederland. (The types of tubercle bacteria in mammals in the Netherlands). Verslagen Tuberculose Studie-Commissie **15**, 59, 1941.

The natural store for the so-called „human type" of the tubercle bacteria exists in human beings, for the bovine type in cattle and for the avian type in poultry. If conditions are favourable, these types may also cause tuberculosis in other kinds of animals. The bovine type proves to be the least specific. Outside their natural medium the tubercle bacteria usually retain their characteristics for a long period (ex.: bovine bacteria from sputum from a woman, who had been suffering from tuberculosis for thirty years). There are however exceptions (ex.: from a veterinary surgeon with bovine infection, after 27 years a strain was cultivated from a fistula which immediately grew eugonically as a human one, but had wholly retained the bovine virulence).

ONG SIAN GWAN, De remmende werking van metalen op den groei van tuberkelbacillen. I. Arseen, antimoon en bismuth. (The inhibitory action of metals on the growth of tubercle bacteria. I) Versl. Ned. Akad. v. Wet. **53**, 345, 1944.

Arsenic, antimony and bismuth inhibit the growth of tubercle bacteria. The inhibitory action of antimony and bismuth exceeds that of arsenic. Bismuth preparations of different purity give identical results. The difference in the inhibitory action of arsenic, antimony and bismuth in a determined concentration is in accordance with the relative position of the elements in the system of elements.

ONG SIAN GWAN, De remmende werking van metalen op den groei van tuberkelbacillen. II. Bismuth. (The inhibitory action of metals on the growth of tubercle bacteria. II. Bismuth). Versl. Ned. Akad. v. Wet. **53**, 353, 1944.

Tubercle bacteria tolerate bismuth; after a protracted period they are not killed, growth continues. They can be transmitted

on fresh media and they may infect guinea-pigs. It is possible to slow down the growth rate of tubercle bacteria or even to inhibit growth altogether by adding bismuth during growth. The inhibitory action of bismuth on tubercle bacteria results from the absorption of the metal by the bacteria.

J. D. Verlinde, De complementbindingsreactie van antistof tegen tuberculeus weefsel met een antigeenmengsel bestaande uit tuberculeus weefselextract en tuberculine. (The complement fixing reaction of antibodies against tuberculous tissue with a mixture of antigens, consisting out of tuberculous tissue extract and tuberculin). Verslagen Tuberculose Studie-Commissie **17**, 98, 1942.

The serum of rabbits injected once subcutaneously with either a boiled saline extract of tuberculous bovine tissue or the same extract unboiled, but filtered through a Seitz filter, or tuberculin, or a mixture of both emulgated in vaselin-lanolin, already after 7 days contains complement fixing antibodies against tuberculin. With a mixture of both antigens in 72 % of the cases more, often even considerably more complement units are fixed than would have been the case after separate appliance of the antigens (Ong's phenomenon).

Serum	Immunisation with	Bound complement units			
		Antigen			
		K	T	KT	KT — (K+T)
Rabbit 1092	Boiled tuberculous bovine tissue extract	10	0	125	+ 115
„ 1093	idem	15	0	150	+ 135
„ 1212	Seitz-filtrate tuberculous bovine tissue	75	0	100	+ 25
„ 1213	idem	75	0	75	0
„ 1172	tuberculin	75	10	125	+ 40
„ 1173	idem	0	10	20	+ 10
„ 1216	idem	75	0	75	0
„ 1217	idem	75	0	75	0
„ 1151	Boiled tuberculous bovine tissue extract + tuberculin	125	10	250	+ 115
„ 1165	idem	15	0	250	+ 235
„ 1214	Seitz-filtrate tuberculous bovine tissue + tuberculin	100	0	125	+ 25
„ 1215	idem	250	0	500	+ 250
Normal		0	10	30	+ 20
„		0	0	0	0

J. D. VERLINDE, Over de pathogene werking van mycobacteriën. (On the pathogenic action of mycobacteria). Verslagen Tuberculose Studie-Commissie **18, 3, 1943.**

The mycobacteria as far as they are reckoned among the acid proof saprophytes and the bacillus of JOHNE are hardly or not at all pathogenic for the usual test animals. As far as they possess some pathogenic properties they can evoke in rabbits and guinea-pigs histological changes reminding of tuberculosis which are limited to the injection spot; some generalisation is rare. Dead tubercle bacilli have similar properties. When, however, the former saprophytes living or dead and the dead tubercle bacilli are injected with fat or oils, generalisation with a proliferating character does ensue. As all mycobacteria are able to evoke essentially the same change, it is probable that they possess pathogenic constituents in common. The virulence might be dependent on their natural covering with lipoids which evidently not in all mycobacteria are equivalent in activity against the body's natural means of resistance.

J. D. VERLINDE, Over de antigene eigenschappen van tuberculeuze weefsels en hun verband met tuberculine-allergie. (On the antigenic properties of tuberculous tissues and their connection with tuberculin allergy). Verslagen Tuberculose Studie-Commissie **19, 17, 1944**

1. The antigenous action of the tuberculous caseous material, „cheese", is for the greater part not specific for the species, for a minor part it is.

2. Tuberculous „cheese" of man, cattle, goat, pig, and rabbit are serologically identical, or they at least contain serologically identical groups. Tuberculous tissue of horse and dog is divergent in this respect.

3. In the serum of normal rabbits and horses, sometimes in that of cattle anti-bodies against tuberculous horse tissue, not against tuberculous „cheese" of cattle can be detected.

4. Neither „cheese"-antigen nor „cheese"-antibodies can as a rule be traced in the blood of the tuberculous animal. Yet, the tuberculous animal is able to form „cheese"-antibodies after having been given „cheese"-antibodies parentererally. Antigen and antibodies are either included in the tuberculous process or they are present in the blood in such a small quantity that they cannot be detected by the usual serological reactions. The fact that the entire individual is sensibilized speaks for the latter view.

5. Guinea-pigs may give a positive reaction of MANTOUX after having been sensibilized with „cheese"-antigen, with a mixture of „cheese"-antigen and tuberculin and sometimes also with tuberculin alone by injecting these substances emulgated in vaselin-lanolin subcutaneously. Their action on the skin is more distinct

if instead of tuberculin a mixture consisting of equal parts of tuberculin and „cheese"-antigen is injected subcutaneously. After the sensibilizing injection has been repeated about six weeks later or perhaps also after a very large dose of „cheese"-antigen, the sensibilization is sufficiently strong to cause a distinct reaction of MANTOUX with tuberculin only.

6. The tuberculin reaction is supposed to be brought about by the formation of a complex toxically composed of an agglutination of antibodies against the tuberculous cell („cheese"-antibodies) to the antigen of the tuberculous cell („cheese"-antigen) and tuberculin. The fact that the tuberculous process sensibilises the entire individual explains that through the formation of such a complex the skin reaction takes place in the skin, the focus reaction in the tuberculous process and the general reaction throughout the entire body.

J. D. VERLINDE, Over het voorkomen van tuberkelbacillen en anaërobe micro-organismen in tandenborstels. (On the occurrence of tubercle bacteria and anaerobic micro-organisms in tooth-brushes). T. voor Tandheelkunde **48**, 779, 1941.

In the long run a mass, consisting of foodrests, tissue-detritus and tooth-paste accumulates at the base of the hairs of a tooth-brush; aerobic and anaerobic mouth bacteria will thrive here. In the tooth-brush of a sufferer from lung tuberculosis living tubercle bacteria were found; in three of four other tooth-brushes anaerobic micro-organisms (*Fusiformis, Actinomyces, B. melaninogenicum, Leptothrix lanceolata*) which are possibly of importance for the affection of mouth and teeth, were detected. Therefore it is important for a good hygiene of the mouth to pay special attention to the hair base when cleaning tooth-brushes.

J. VAN DER HOEDEN, Tuberkelbacillen in zuivelproducten (Tubercle bacilli in dairy products). Verslagen Tuberculose Studie-Commissie **14**, 77, 1940.

The examination of dairy products, prepared from non-heated milk in the country produced the following results:

a. Two out of 15 samples of butter milk contained living tubercle bacilli of the bovine type. They originated from stables where the cattle were suffering from tuberculosis.

b. In none of 72 samples of whey-butter tubercle bacilli were found.

c. In 2 out of 24 samples of farm-butter tubercle bacilli of the bovine type were found. These 2 samples had been prepared from: 1. milk from cows from various farms which had been milked in the cattle market; 2. milk from a stock of cattle where serious tuberculosis was prevalent.

d. Bovine tubercle bacilli were found in one out of 56 fat cheeses

originating from 42 farms. The infected cheese had been made 4 weeks earlier at a farm where the cattle had been infected in a high degree with tuberculosis for a considerable time. Three months later living tubercle bacilli could no longer be ascertained in that cheese.

In order to test the resistance of tubercle bacilli in dairy products, fat cheese had been made from milk to which had been added 1 : 100 milk from a cow with tuberculosis of the udder which contained many tubercle bacilli, though it did not show any macroscopic alterations. Then butter was prepared from a mixture of the same milk in the ratio 1 : 20. In the thus obtained butter milk, which was kept at room temperature, the tubercle bacilli proved virulent after 32 days (pH = 3.6). In the salted butter kept at 4—6° C. they had still retained their virulence after 151 days; after 180 days living, though less virulent bacilli were still found; after one year no more could be detected. In the fat cheese kept at room temperature during 82 days and subsequently at 4—6° C., the tubercle bacilli after one year were still present in large numbers and at high virulence. After 17 months they had died off.

So dairy products prepared from non-heated milk may constitute a source of danger to human health. This danger is most imminent in the country, where these products are consumed by a small group of individuals and so may cause ever recurring infections with all the evil consequences of this. The problem of the danger inherent in the consumption of dairy products containing tubercle bacilli deserves full consideration. For concerns which manufacture dairy products from non-heated milk it would be worth considering to ask for a guarantee that the cattle is free from tuberculous infection or to have pasteurisation of the milk made obligatory.

J. H. Bekker, A new acid-fast bacillus isolated from a patient, suspected to be suffering from tuberculosis. Antonie van Leeuwenhoek **9**, 81, 1943. Cf. also: Een zuurvaste bacterie geïsoleerd uit een van tuberculose verdachten patient. Verslagen der Tuberculose Studie-Commissie, **18**, 18, 1942.

From the catheter urine of a patient, the right kidney of whom had been removed at an earlier date in connection with a clinically diagnosed tuberculosis, an acid-fast bacillus was isolated. On a closer investigation this bacillus appeared to belong to the group of the so-called saprophytic acid-fast bacilli, but its properties are different from all acid-fast bacteria described hitherto. The newfound bacillus was called *Mycobacterium Bekkerii*.

J. H. Bekker, De intercurrente sterfte van caviae bij het onderzoek op tuberkelbacillen. (The intercurrent mortality of guineapigs during the tracing of tubercle bacteria). Geneeskundige Gids **22**, 171, 1944.

In an investigation of the intercurrent mortality of guinea-pigs during the tracing of tubercle bacilli, it appeared that the mortality was caused in a merely slight degree by the injected material. Climatic and diethetic conditions play a far greater part. Some measures which might induce a change herein are discussed.

J. D. VERLINDE, The sensitiveness of the guinea-pig and the rabbit for *Mycobacterium Bekkerii*. Antonie van Leeuwenhoek **9**, 129, 1943.

The guinea-pig and the rabbit may be infected by a acid fast micro-organism, isolated by BEKKER. After intraperitoneal and subcutaneous inoculation, a granulation tissue, especially in the liver is excited, which can hardly be distinguished from proliferative tuberculosis as found in bovine tuberculosis in horses and avian tuberculosis in swine.

J. D. VERLINDE, Experimenteel onderzoek naar de pathogene werking van den Bacil van BEKKER. (Experimental investigation of the pathogenic action of *Mycobacterium Bekkerii*). Verslagen Tuberculose Studie-Commissie **18**, 34, 1943.

Mycobacterium Bekkerii is by its nature pathogenic for the guineapig. After subcutaneous and intraperitoneal injection generalisation occurs. A definite influence of paraffin on the virulence can be noted. Dead B.C.G. is but slightly pathogenic and causes at most a local process, speadily healed. Paraffin may evoke a generalised disease at least after peritoneal injection.

The bacillus of JOHNE can be pathogenic for the guinea-pig and even cause slight generalisation (small nuclei in liver and spleen). When paraffin is used the generalisation becomes distinct. The increase in the density of the dissimination shows best in the liver, which teems with miliary and submiliary small nuclei, which often are confluent. After injection of dead B.C.G. in paraffin, however, nuclei are met with only sporadically. This might indicate that living bacteria (*Mycobacterium Bekkerii* and the bacillus of JOHNE) will multiply in the guinea-pig. The paraffin would act then in two ways: 1. the transfer of the bacteria throughout the body; 2. the protection of the bacteria against the means of defence of the host, so that they might develop better in its presence. Presumably the acid proof saprophytes which still dispose of some pathogenic property are also acted upon in both ways. So the infection and along with it the virulence is increased.

A *Mycobacterium* already virulent by its nature such as *Mycobacterium Bekkerii* and probably the tubercle bacillus does not need the paraffin. Such bacteria may possess in their capsules the necessary lipoids which are as strongly protective as paraffin. In its histological aspect the investigation substantiates the con-

ception that many mycobacteria possess a definite pathogenic property in common, evoking a same type of proliferative inflammation, characterised by the occurrence of epitheloid cells, giant cells and lymphocytes.

W. A. P. Schüffner en H. Bohlander, Bacteriologisch en epidemiologisch onderzoek van modderkoorts (Schlammfieber) in Nederland. (Bacteriological and epidemiological investigation of mire-fever (Schlammfieber) in the Netherlands). Ned. T. voor Geneeskunde **85**, 4390, 1941.

In two boys, who had gone out in pursuit of voles, mire-fever was found, a leptospirosis up till then not observed in the Netherlands. The underlying leptospira, *L. grippotyphosa*, was demonstrated in the kidneys of the common vole, *Microtus arvalis* (Pallas). It still remains to be proved, whether this disease only occurs in the Rhine-Valley near Huissen or whether in other regions of this country too certain morbid phenomena should suggest the possibility of mire-fever.

W. A. P. Schüffner en H. Bohlander, Voortgezette waarnemingen over modderkoorts in Nederland, een laboratoriuminfectie. (Continued observations of mire-fever in the Netherlands, a laboratory infection). Ned. T. voor Geneeskunde **86**, 1341, 1942.

A laboratory infection with mire-fever is described, occurring after a bite of a wild vole whose urine contained innumerable leptospirae. The illness ran a high fever, ending by crisis and followed by a brief recurrence. *Leptospira grippotyphosa* could be demonstrated in the patient's blood on the first day of illness, but no more in the blood withdrawn during the last (5th) fever attack. In contradiction with the known facts, in this case again the leptospira appeared in the urine, in which after two months it could still be demonstrated intermittingly.

W. A. P. Schüffner und H. Bohlander, Die ersten Ergebnisse der Schlammfieberforschung in den Niederlanden. (The first results of the investigation of the mire fever in the Netherlands). Antonie van Leeuwenhoek **9**, 19, 1943.

For more than a year the occurring of mire fever in the Netherlands has been investigated. In that period 21 cases have been reported. From 479 examined mice 146 (31 %) were carrier of *Leptospira grippotyphosa*. The spreading is not universal; it fluctuates from 1—100 % according to place and time.

P. Muntendam, W. A. P. Schüffner en H. Zeisling, Modderkoorts in Friesland. (Mire fever in Friesland). Ned. T. voor Geneeskunde **87, 397, 1943.**

In central Friesland, where a strong epizoötia with *L. grippotyphosa* was found among voles, 9 cases of mire fever have been diagnosed. The patients are exclusively farm labourers, who were occupied with the raking and cutting of grass.

W. A. P. Schüffner und H. Bohlander, Über eine Epizootie unter zahmen Ratten und die dagegen gerichtete Schutzimpfung des Personals. (On an epizootic among tame rats and on the active immunization against it of the staff). Antonie van Leeuwenhoek **7, 1, 1941.**

Among the staff of a big commercial laboratory a case of Weil's disease occurred. The infection was supposed to be caused by the bite of a tame rat. This animal formed part of a group of 50 rats. Among these 15 leptospira carriers were found. From the remaining 3000 rats a random test was carried out with 42 animals; 18 leptospira carriers were found. Consequently on an average 36% of the adult animals were infected. The staff of the laboratory was immunized actively by administering the home-made vaccin two times intravenously with an interval of a week; the first time 1 ml, the second time 3 ml.

P. H. van Thiel, Het gebruik van liquoid (Roche) in de practijk van de diagnostiek der ziekte van Weil. (The use of liquoid (Roche) in the diagnostical practice of the Weil's disease). Ned. Tijdschr. voor Geneeskunde **85,** 3018, 1941; Cf. also: Acta Leidensia **15—16,** 328, 1940—1941.

It is possible to demonstrate leptospirae in blood collected in 1 % liquoid (Roche) in normal saline, by double centrifuging. The addition of acid buffer fluid, as done in Ruys' double centrifuge method, is impossible. When carried out within 3 days after collecting the blood, the sodium oxalate method of Ruys is preferable to the liquoid method. When the centrifuging process cannot be done before 3 days after taking the blood, it is better collected in liquoid. If it is collected too long ago, one must put up with one-stage centrifuging. Under equal circumstances the second centrifuging can be carried out during a longer period with blood stored up with liquoid than with oxalate blood. Both in temperate zones and in the tropics it is possible to isolate leptospirae by animal experiment from liquoid or oxalate blood up to at least 6 days after forwarding to laboratories.

P. H. VAN THIEL, Ist es möglich *Leptospira icterohaemorrhagiae* durch Kulturversuchen aus Oberflächenwasser zu isolieren? (Is it possible to isolate *Leptospira icterohaemorrhagiae* out of surface water?) Antonie van Leeuwenhoek **7**, 137, 1941.

Because the antagonism described by APPELMAN and VAN THIEL between the pathogenic *Leptospira icterohaemorrhagiae* and *Leptispora biflexa* under definite conditions does not occur in the egg agar medium of ZUELZER, it has been tried to isolate by means of this nutrient medium *Leptospira icterohaemorrhagiae* out of surface water, in which merely a small number of Leptospirae occur. Although the water under examination had been supplied with numerous Leptospirae, it has not been possible to detect their presence by means of injection of this heavily contaminated water in guinea-pigs. Neither has it been possible by means of a combination of the filtration method of VINZENT and the egg agar method of ZUELZER. As *Leptospira icterohaemorrhagiae* as yet cannot be cultivated from surface water, the methods described by the author have to be applied.

P. H. VAN THIEL und W. L. C. VEER, Biologische Methoden zum Isolieren von *Leptospira icterohaemorrhagiae* aus Wasser. (Biological methods for the isolation of *Leptospira icterohaemorrhagiae* from water). Antonie van Leeuwenhoek **7**, 221, 1941.

It has been tried to improve the bathing method of APPELMAN which aims at the isolation of pathogenic Leptospirae from surface water. Three new biological methods have been described: 1. a centrifugation method, 2. a flooding of the guinea-pig, 3. a subcutaneous transflooding of the guinea-pig. The 2nd and 3rd method are recommended. By these means it is possible to detect a single Leptospira in 50 ml of water. When compared with the bathing method, the latter methods offer the following advantages: *a*). a 9—19 times greater possibility to detect pathogenic Leptospirae in surface water; *b*). a smaller number of guinea-pigs needed for the experiment.

L. DE BLIECK and JAC. JANSEN, Listerellose bei Tieren. A. Listerellose bei Ferkeln. (Listerellosis in animals. A. Listerellosis in pigs). Antonie van Leeuwenhoek **9**, 93, 1943. Cf. also: L. DE BLIECK en JAC. JANSEN, Listerrellose bij biggen. (Listerellosis in pigs). Tijdschrift voor Diergeneeskunde **69**, 573, 1942.

Out of 5 days old pigs a bacterium was isolated that proved to be a *Listerella*. In the livers of the pigs multiple necrosis was found. The bacterium was pathogenic for pigs, rabbits, mice, rats and guinea-pigs and extremely pathogenic for canaries. The same changes in the liver were observed in experimentally infected rabbits, rats and mice. In the pig and the rabbit encephalitis could

be provoked. The strain proved to be identical with a *Listerella*-strain isolated by KAPSENBERG from a child that had died of encephalitis.

JAC. JANSEN und C. F. G. W. VAN DER HURK, Listerellose bei Tieren. B. Listerellose bei der Ziege. (Listerellosis in animals. B. Listerellosis in the goat). Antonie van Leeuwenhoek **9**, 104, 1943.

From the mesenteric lymph glands of a goat, having died as the result of a hemorrhagical necrotising inflammation of the large intestine, a *Listerella* was isolated, identical with a *Listerella* strain isolated from pigs.

A. CH. RUYS, De waarde van typebepaling voor het epidemiologisch onderzoek bij diphtherie. (The value of the determination of type for the epidemiological investigation of diphtheria). Ned. T. voor Geneeskunde **84**, 3969, 1940.

In the autumn of 1939 and the spring of 1940 diphtheria in Amsterdam was nearly exclusively caused by the mitis type. In a few cases connected epidemiologically an atypical gravis strain was grown, corresponding with a strain isolated by SIEMENS in 1936. In the same period in a rigidly secluded group of Jewish children from Germany an outbreak prevailed caused by the normal gravis type. Two groups of carriers, later shown to carry different types, for some months lived together in a institution. In 16 out of 54 children a change of type was observed. It is undesirable that diphtheria carriers should be nursed together, as the carrier period can be lengthened by reinfection.

A. L. NOORDAM, Een diphtherie-epidemie, veroorzaakt door het gravis type van *Corynebacterium diphtheriae* in een kindertehuis te Amsterdam. (An epidemic of diphtheria caused by the gravis type of *Corynebacterium diphtheriae* in a children's home at Amsterdam). Ned. T. voor Geneeskunde **84**, 3962, 1940.

Report of an outbreak of diphtheria caused by the gravis type of *C. diphtheriae* in a children's home, from which the children could not be evacuated any more. Of 96 children, 26 contracted the disease, 19 of whom had received complete immunisation 4 months before. Of 8 patients the SCHICK reaction had been found negative just before the onset of the illness. 30 children were isolated as carriers. The value of immunisation is discussed. The possible usefulness of the „injection de rappel" is pointed out.

Ch. G. J. Dornickx en J. F. Hulk, Bacteriologische diphtherie diagnostiek door middel van de Clauberg III plaat, een telluur-indicatoren-plaat welke reeds bij macroscopische aflezing aanwijzingen geeft. (Bacteriological diagnostics of diphtheria by means of the Clauberg III plate, a tellurite-indicator-plate, which furnishes macroscopic indications). Geneeskundige Gids **19**, 719, 1941.

The Clauberg indicator plate already by macroscopical readings furnishes important indications for the bacteriological diagnostics of diphtheria. The properties of the indicator plate are discussed and compared with those of the Loeffler serum medium. The results are reported of a comparative test covering 7116 samples, wherein the Loeffler and the indicator plate have been used side by side. The indicator plate appeared to indicate a number of positive cases only slightly higher than the Loeffler plate. The use of both plates together, however, gave a large increase of positives.

A. P. van der Wey, De prognose van diphtherie, in verband met het bacterietype. (The prognosis of diphtheria in connection with the type of bacteria). Ned. T. voor Geneeskunde **87**, 203, 1943.

The malignant forms of diphtheria are caused more often by the gravis type than by the mitis type. The latter is more laryngotrope than the former. The intermedius type seems to resemble the gravis type more closely than the mitis type.

A. Charlotte Ruys, On the behaviour of *E. typhi* in surface water. Antonie van Leeuwenhoek **7**, 93, 1941.

Several typhoid infections were traced to direct or indirect contact with contaminated water from which typhoid bacteria could be isolated. Especially during the winter months typhoid bacteria could be isolated from surface water. In experiments the disappearance of typhoid bacteria from contaminated water is accelerated much more by the influence of light than by the action of protozoa. The definite phototaxis of typhoid bacteria may cause the rapid decrease of the microbes from the depth of surface water as well.

K. C. Winkler en W. J. Quarles van Ufford, Typhusgevaar door het eten van aardbeien en sla. (Danger of typhoid through the eating of strawberries and salad). Ned. T. voor Geneeskunde **85**, 1200, 1941.

The importance of strawberries and salad as vehicles of typhoid for the spreading was studied by examining their infestation with

coli bacteria as an indicator of faecal contamination. It was found that: *a*. On strawberries nearly always coli bacteria are found, on salad in general they are absent; *b*. On both products coli bacteria remain alive for at least 24 hours; *c*. The juice of strawberries has a growth inhibiting action, that of salad has none; *d*. By washing with water coli bacteria are not removed, but their number is diminished; *e*. Strawberries and salad can be efficiently desinfected with 1 % hydrochloric acid (salad also with vinegar) without loss of flavour. The common salad sauces cannot be relied upon for this purpose.

G. D. Hemmes, Overbrenging van typhus door kaas. (The transmitting of typhoid bij cheese). Ned. T. voor Geneeskunde **87,** 203, 1943.

Description of the coming down with typhoid of 8 persons, from eating cheese. 40 days after the making of this cheese, typhoid bacteria could be grown from it.

B. ten Have en A. P. van der Wey, Over het kweeken van typhusbacteriën uit bloed en beenmerg. (On the culturing of typhoid bacteria out of blood and bone marrow). Ned. T. voor Geneeskunde **86,** Noodnummer VI, 57, 1944.

Next to the culturing of typhoid bacteria out of the blood the authors isolated these bacteria also from bone marrow by means of punction of the sternum (0.5 cc marrow punctate added to 10 cc ox bile). The bacteriological test of the bone marrow offered positive results during a period of more than a week when the blood was already negative; it may thus be of value in the phase when the blood is already negative and the culturing out of feces and urine not yet positive.

A. Clarenburg, *Salmonella aberdeen* gekweekt uit een voedingspreparaat. (*Salmonella aberdeen* cultured from a nutrient preparation). Geneeskundige Gids **18,** 1040, 1940.

The investigation of a strain of paratyphoid cultured from a nutrient preparation is described. The strain appeared to belong to the type *Salmonella aberdeen*. The 3 children of the family from which the nutrient preparation had been derived, suffered from paratyphoid. In the feces of these patients *Salmonella paratyphosum* B has been detected. A causal connection between the occurrence of the disease symptoms and the partaking of the preparation could not be established. The pathogenicity of *Salmonella aberdeen* for men is not established as yet.

H. H. Vink, R. Th. Scholtens en C. A. van Hees, Een geval van infectie met *Salmonella montevideo*. (A case of infection with *Salmonella montevideo*). Ned. T. voor Geneeskunde **87, 1433, 1943.**

Description of a case of paratyphoid fever caused by *Salmonella montevideo* (antigen formula VI, VII, g, m, s).

A. Ch. Ruys, Erváringen met den voedingsbodem van Leifson voor het isoleeren van dysenteriebacillen. (Experiences with the nutrient medium of Leifson for the isolation of dysentery bacilli). Ned. T. voor Geneeskunde **84, 3562, 1940.**

For the detection of bacillary dysentery by examination of the feces the Leifson culture medium is indispensable. This medium must always be used freshly prepared, and subcultures must be taken after 24 and 48 hours' growing at 37° C.

E. A. Tillema, Een oriënteerend onderzoek over den voedingsbodem van Leifson voor bacteriëele dysenterie. (A preliminary investigation of the nutrient medium of Leifson for bacterial dysentery). Ned. T. voor Geneeskunde **84, 3564, 1940.**

Some results are recorded concerning the cultivation of pathogenic bacteria of the intestines on the sodium-desoxycholate citrate plate of Leifson. This plate proved to be pre-eminently suited for dysentery bacteria, not so however for the group of the paratyphoid and typhoid bacteria.

R. Th. Scholtens, In Nederland waargenomen gevallen van dysenterie, veroorzaakt door *B. dysenteriae* New Castle. (Cases of dysentery caused by *B. dysenteriae* type New Castle noted in the Netherlands). Ned. T. voor Geneeskunde **88, 242, 1944.**

Bacteriological and serological description of cases of dysentery observed in the Netherlands, caused by *B. dysenteriae* type New Castle (agreeing with the type 88 of Boyd).

R. Th. Scholtens, Een onbekend type dysenteriebacterie als oorzaak eener epidemie. (An unknown type of dysentery bacterium as the cause of an epidemic). Ned. T. voor Geneeskunde **84, 1613, 1940.**

It was proved that an outbreak of 20 cases of dysentery was caused by a type of dysentery bacterium unknown till the present. This type in view of its biochemical properties belongs to the group of lactose-fermenting dysentery bacteria. Serologically it takes a separate position.

D. L. Hulst, Onderzoekingen over bacillaire dysenterie in Leiden en omgeving. (Investigations of bacillary dysentery in Leiden and its neighbourhood). Thesis, Leiden 1940.

A survey is given of the history, the clinic, the epidemiology and the bacteriology of *B. dysenteriae*. The author arrives at the conclusion that *B. dysenteriae* Sonne occurs endemically in Leiden and its neighbourhood and probably in all of the Netherlands. This conclusion is substantiated by the occurrence of high agglutination titers against *B. dysenteriae* Sonne in sera of persons in normal health. These agglutinins are deemed by the author as true rest agglutinins.

C. D. Westermann, Over het voorkomen van *Shigella dysenteriae* type New Castle en het kweeken van dysenterie bacteriën uit de faeces. (On the occurrence of *Shigella dysenteriae* type New Castle and the culturing of dysentery bacteria from feces). Ned. T. voor Geneeskunde **88**, 242, 1944.

The author isolated at Amsterdam from feces of patients in 3 cases *Shigella ambigua* and in 108 cases *Shigella dysenteriae* type New Castle. The suitability of the medium of Leifson for the diagnosis of bacillary dysentery is emphasized again.

J. D. Verlinde en H. D. Boer, De verwekker van hepatitis epidemica. (The cause of hepatitis epidemica). Ned. T. voor Geneeskunde **87**, 1304, 1943.

In four patients suffering from hepatitis epidemica the authors succeeded in isolating a filterable virus from the blood during the period of fever and from the urine during the icteric stage. The virus is pathogenic for the guinea-pig after intraperitoneal, intracardial, subcutaneous and intracerebral inoculation. The only morbid symptom in the guinea-pig after an incubation time of ± 7 days, is a fever period of some days. In the animals the virus can be shown during the fever period in the blood and in the liver. After the period the virus is excreted with the urine apparently for a short time. In the livers of the guinea-pigs areas of fatty degeneration, dissociation and hyalin necrobiosis may be found. After pulling through the disease immunity occurs, whilst in the serum of convalescent men and guinea-pigs neutralizing antibodies can be shown.

J. D. Verlinde and A. J. van den Hoven van Genderen, A filterable virus as a causative agent of epidemic hepatitis. Antonie van Leeuwenhoek **10**, 29, 1944—1945.

In four patients suffering from epidemic hepatitis we succeeded in isolating from the blood during the fever period and from the urine during the jaundice, a filterable virus, which is pathogenic

to the guinea-pig and which can be inoculated into this animal in different ways, but by preference intraperitoneally. Fever which sometimes lasts only one day is the only morbid symptom observed in guinea-pigs. In these animals the virus can be shown in the organs and in the blood during the fever period; after that it is excreted with the urine during apparently a short period. The virus has been grown on the chorioallantois of the chick embryo so far during 20 passages. The virus is resistent to glycerol, drying and low temperatures, not to formaline and heating. In the livers of the guinea-pigs focal changes (degeneration, dissociation, necrobiosis, yellow liver-atrophy) may be found. After pulling through the disease immunity occurs, whilst in the serum of recovered patients and guinea-pigs neutralizing antibodies can be detected.

J. van Lookeren Campagne, Pneumococcus-onderzoekingen bij kinderen. (Investigations of pneumococci in children). Ned. T. voor Geneeskunde **85**, 622, 1941.

The results are communicated of a type investigation in 157 cases of pneumococcal infection in the Groningen Children's Hospital observed during the years 1938—1941. After type 1, preponderating mainly as a causative organism of empyema, type 6 follows in frequency; the types 19, 23, 12, 7, 18, 3 and 14 were also comparatively frequent. The practical importance of the type-examination, though having diminished since the introduction of sulfapyridine, remains considerable for the combined chemo- and serotherapy of meningitis, septic pneumonia and peritonitis.

J. Mulder, Pneumococci-types bij pneumococcieën van volwassenen in Nederland. (Types of pneumococci in pneumococcises of adults in the Netherlands). Ned. T. voor Geneeskunde **85**, 692, 1941.

From March 1935 to September 1940 in Holland 560 pneumococcus strains were typed (Neufeld method) from various pneumococcal infections in adults (over 12 years). Type 33 of Etinger Tulczynska (related with type 19) occurs rather frequently in the bronchitis group and in otitis media. In lobar pneumonia and empyema type 1 takes the first place with 58.3 % as a causal organism. Then follows the types 2 (12 %), 7 (9 %), 3 (9 %), 5 (6.5 %), and 4 (2.5 %). The higher types of Cooper are practically of no significance in lobar pneumonia. In meningitis the types 1 and 3 prevail, but higher types are relatively frequent. In bronchopneumonia and bronchitis type 1 is absent; types 2, 3 and 6 are rather frequent here, and so are the higher types of Cooper. 7.3 % of the strains could not be typed. The types 25, 27 and 32 did not occur among the 560 strains.

J. H. Bekker, Botulisme in Hees. (Botulism at Hees). Ned. T. voor Geneeskunde **88,** Noodnummer VII, **69, 1944.**

Report of the bacteriological investigation of an epidemic of botulism at Hees, caused by the eating of smoked pork. 58 persons partook of this food, 24 fell ill out of which 9 died. The isolated toxin was not neutralized by the anti-botulinus sera A or B; other anti-botulinus sera were not available. The possibility that here one of the other types or a new type may have caused the epidemic is left open.

J. H. Bekker, Botulisme in Nederland. (Botulism in the Netherlands). Geneeskundige Gids **22,** 303, **1944.**

After a description of the bacteriology, the epidemiology, the clinic, the therapy and the prophylaxis of botulism all epidemics which have become known in the Netherlands are reviewed. Since 1899 17 epidemics of botulism have become known, in 8 the clinical diagnosis has been confirmed bacteriologically, in the others the diagnosis has been merely clinical. All together the epidemics bear on 76 patients, out of which 21 died, thus an average letality of 27 %, whilst the letality of the various epidemics varied between 0 to 100 %.

J. J. van Loghem, The classification of the plague-bacillus. Antonie van Leeuwenhoek **10,** 15, **1944—1945.**

The author proposes to classify the plague-bacillus, together with the nearly related rodentium bacillus, in a new genus *Yersinia* (*Y. pestis*, *Y. rodentium*) and to place this genus with other unclassifiable genera into the family of the *Bacteriaceae.*

R. Abdoelrachman, Vibrio research in the Hejaz in connection with the El Tor problem. Antonie van Leeuwenhoek **10,** 93, **1944—1945.**

Water vibrios occurring in many water sources can easily be differentiated from *V. cholerae*. Several times *V. cholerae*-like vibrios were isolated from the stools of people suffering from gastroenteritis.

In 1905 Gotschlich and afterwards other investigators as well isolated at El Tor from the feces of pilgrims who were not suffering from cholera, vibrios which according to some of them were identical with the actual *V. cholerae*, but which in the opinion of others must not be considered as such. In 1937—1938 at Macassar and its surroundings from the stools of people suffering from cholera-like gastroenteritis, as well as from waters, which were probably polluted with the feces of those people, de Moor isolated germs

which in every respect were like the El Tor vibrio. Finally the author in 1937—1938, in a water sample derived from the Zam-Zam well at Mecca (Hejaz), the water of which is drunk by thousands of pilgrims without any harmful effect, detected germs which were absolutely identic with *V. El Tor* of GOTSCHLICH. The El Tor vibrio is never found in the stools of people bound for the Hejaz, but several times in the feces of home-bound pilgrims.

These facts seem to warrant the following conclusions: *a. V. El Tor* forms a group of vibrios of its own, neither identical with *V. cholerae* nor with water vibrios. *b.* This group of vibrios may be subdivided into 2 types: 1. The apathogenic type which is found in the Hejaz and surroundings; this vibrio may be called: *Vibrio El Tor*, type Hejaz. 2. The pathogenic type, which is found at Macassar and surroundings; this may be called: *Vibrio El Tor*, type Macassar.

B. TEN HAVE, Onderzoekingen over de groep der kapselbacteriën, in het bijzonder in verband met de ozaena. (Investigations of the group of capsulated bacteria, more especially in connection with ozena). Thesis, Amsterdam 1943.

Recent literature dealing with the system of the Klebsiellae suggests that difficulties may arise, when it comes to separating the genus *Klebsiella* from other genera, particularly from *Aerobacter* and *Citrobacter*; the grouping of the species is likewise fraught with difficulties. All the same the opinion is gaining ground, that there is room for the species *Kl. pneumoniae* and *Kl. ozaenae* besides the well-defined species *Klebsiella rhinoscleromatis*.

383 capsulated strains of *Klebsiella* have been examined, 24 belonging to *Kl. rhinoscleromatis*, 23 to *Kl. pneumoniae*, 330 to *Kl. ozaenae* and 6 to *Kl. paulum fermentans*. Provisionally, the author has introduced *Klebsiella paulum fermentans* as a new species. With the 330 strains of *Kl. ozaenae* (of which 3 belonged to the capsulated type C, 294 to D, 3 to E, 19 to A, 5 to E, 3 to F and 3 to the remaining group Y) it was possible, without exception, to distinguish them from the other species; the same also applied to the 25 strains which were certainly not of ozenal origin. For the identification of the *Klebsiella* the author considers the agglutination of the decapsulated ¢-variant of paramount importance. However, the finding of an efficient method of decapsulation has failed. It is taken that the age of the strains plays an important part in the appearance of ¢-variants. In most cases the ¢-variants (ectoplasma polysaccharide free) immediately originated from the K-form; sometimes, however, an ¢ form (decapsulated but with ectoplasma still containing some polysaccharide) was intercalated as an intermediate stage. The characters of the 54 ozena ¢-variants collected led to the establishment of a preliminary division into 4 types on the basis of agglutination.

In 300 ozena patients capsulated bacteria have been found 248

times. By far the majority harboured *Kl. ozaenae*, 10 showed *Kl. pneumoniae* and 4 *Kl. paulum fermentans*; *Kl. rhinoscleromatis* was never met with. In cases of ozena the most predominant bacteria are the Klebsiellae. The notion that these are closely connected with ozena is supported by the results of the agglutination tests carried out with sera from patients. In ozena the real coccobacillus foetidus of PEREZ has been met with only sporadically; agglutination tests with sera from patients did not tell in favour of a close connection between this bacterium and ozena. *B. proteus* and related organisms are only of importance as the originators of fetor. Corynebacteria found in ozena should be considered as saprophytes. The view is held that there is no sufficient ground to consider any of the microbes found as the fundamental cause of ozena.

I. J. LE COSQUINO DE BUSSY, J. J. VAN LOGHEM en A. K. VISSER, *Staphylococcus aureus* in den gezonden neus. (*Staphylococcus aureus* in the healthy nose). Ned. T. voor Geneeskunde **86**, 2629, 1942.

The authors examined 1559 children and adults for the presence of *Staphylococcus aureus* in the healthy nasal cavity, and obtained in the various groups 36—63 % positive results. The commensal strains of *Staphylococcus aureus* which they could grow were undistinguishable bacteriologically (by examination for pigment, haemolysins, coagulase, toxin and antigenic structure) from strains of *Staphylococcus aureus* from pathologic products.

J. C. VERHAGE en W. HEKMAN, Een epidemie van streptococcus infecties op een chirurgische afdeeling, veroorzaakt door een bacillendrager. (An epidemic of infections by Streptococci caused by a carrier). Ned. T. voor Geneeskunde **87**, 536, 1943.

Description of an epidemic of wound infections in a surgical department, caused by haemolytic streptococci, which started in the throat of a germ carrier. These haemolytic streptococci have caused various clinical pictures, *viz.*, purulent wound infections with an exanthema with no characteristics of scarlet fever, erysipelas, wound scarlet fever and ordinary scarlet fever.

FRANZ PICK, Beobachtungen an einer Kultur von *Entamoeba histolytica* (Schaudinn). (Observations of a culture of *Entamoeba histolytica* (Schaudinn). Antonie van Leeuwenhoek **7**, 13, 1941.

An adequate enrichment method for *Entamoeba histolytica* is liver infusion unto which starch suspended in insulin has been added. The vegetative form of *Entamoeba histolytica* is polarized. The cysts of *Entamoeba histolytica* show as a biological reaction after supply of defribinated sheep blood ring formation on distance.

Thus the diagnosis of „cysts" is made easier. Pyocyanin is a vital stain for *Entamoeba histolytica*; it makes structure and nucleus more clearly visible and increases the motility of the amoebae. Some observations of a twisting of the stem of a pseudopodium give rise to the assumption that an ectoplastic excystation spot exists and that the amoebae do not slip out nakedly.

F. J. A. Paesi, Observations on „healthy" human carriers of *Plasmodium vivax*. Antonie van Leeuwenhoek **10**, 77, 1944—1945. Cf. also: Ned. T. voor Geneeskunde **88**, 670, 1944.

The number of plasmodia in the blood of sufferers of chronic malaria tertiana varies greatly. An examination of the blood of 15 childern carried out from November till June (Sundays excepted) brought to light that these variations occur more or less regularly and that even negative phases may alternate with parasitic recidives. Based on these findings it is advised that for the detection of plasmodium carriers examination has to be followed by a second and third examination after 10 and 12 days.

Pik Ging Hoo, Aangeboren malaria. (Congenital malaria). Ned. T. voor Geneeskunde **85**, 1542, 1941.

The problem of congenital malaria is discussed in connection with a number of observations recorded in literature. A case is described in which the possibility of congenital malaria must be seriously reckoned with.

The child's mother got her first attack one day after delivery. On its 20th day of life malaria parasites were found in the child's blood. In the period during which the child's blood contained malaria parasites, there was at first no fever, afterwards some slight pyrexia; the spleen was not enlarged. The child also had a congenital heart lesion.

S. L. Brug, Exo-erythrocytaire malariaparasieten. (Exo-erythrocytar malaria parasites). Ned. T. voor Geneeskunde **85**, 2745, 1941.

Description of a preparation containing three structures probably exo-erythrocytic plasmodia, obtained from smears of human lung tissue. The patient, suffering from paralysis, had, 10 days before, been inoculated intravenously with blood containing *Plasmodium vivax*. At the time of death, as some days before, benign tertian parasites were found in his blood and he died during the acute stage of malaria. The E-E-forms are reproduced. In smears of the spleen, the liver, the kidney, the bone marrow and the brain no parasites were found.

G. VAN DER MEER en S. L. BRUG, *Pneumocystis*, als parasiet bij den mensch. (*Pneumocystis*, a parasite of man). Ned. T. voor Geneeskunde **86**, 2066, 1942.

The search for plasmodia in a three months old child, who died from malaria, revealed in the smears of the lungs the presence of a considerable number of *Pneumocystis*. In the smears coloured with KIEWIT DE JONGE's stain several cysts of 5—7 μ diameter with 8 elements within could be demonstrated. Also unripe cysts with 4, 2 and 1 nuclei were found. All these forms did not differ from *Pneumocystis carinii* as found in many animal species. Besides these classical cysts the writers describe other structures which they consider to belong to the lifecycle of the parasite: 1. in the smears there were groups of nuclei, each with a more or less ill defined protoplasm, the whole often together with a number of cysts embedded in a pale pink matrix; 2. corresponding with these groups honeycomb-like structures were found in sections of the lungs, each combcell containing a small mass of protoplasm with a minute nucleus. Smears of the lungs of 78 postmortems were thoroughly examined. Only once a similar cyst was found, but this was an unmistakable one. In the lungs of rats and mice, both wild and tame, and in one guinea-pig out of 14 the same cysts were found and also the honeycomb-like structures.

As to the systematic position of the parasite the authors consider it to be uncertain. They are convinced, however, that it is not a coccidium.

J. D. VERLINDE, De actieve medewerking van bacteriën bij tandcaries. (The active co-operation of bacteria in tooth caries). T. voor Tandheelkunde **49**, 506, 1942.

A number of aerobic and anaerobic bacteria have been isolated in pure culture from carious elements; with these it was attempted to cause caries in vitro on sterilized elements. This succeeded for eight kinds of bacteria, among which 2 aerobic (*Streptococcus acidi lactici* and *Diplococcus crassus*) and 6 anaerobic strains (*Fusiformis*, *Vibriothrix tonsillaris*, 3 kinds of *Leptothrix*, *Diplococcus tonsillaris*) in sugar containing substrate but only if the enamal had been pierced before. The enamel was not affected by the bacteria.

J. MULDER, R. VAN DEN BERG en R. VAN KOLLEM, De influenza-epidemie van Februari-Maart 1939 in het garnizoen te Groningen. II. Bacteriologie. (The influenza epidemy of February-March 1939 in the garrison of Groningen. II. Bacteriology). Ned. T. voor Geneeskunde **84**, 2141, 1940.

The 1939 outbreak of influenza in the garrison of Groningen lasted from about February 1st to about March 6th. Of the soldiers 36 % got actually ill. The illness had a very mild course and was

characterized clinically by a pyrexia lasting 1 to 6 days, a dry cough being the main clinical symptom. The number of patients with complications was only 4 %, mainly tonsillitis, otitis media and two cases of bronchiolitis and bronchopneumonia respectively. In 3 of these cases a haemolytical streptococcus type 5 was cultured. In the patient with purulent bronchiolitis *H. influenzae* was found besides. In 4 patients the diagnosis was confirmed bacteriologically by transference on a ferret. Of one patient the ferret strain was transferred on mice. The direct transference of the disease from man to mouse did not succeed, neither that from the first ferret on a mouse. Transference of the first and subsequent passages upon mice succeeded easily. The virulence of this virus strain is at present moderate, about a thousand times weaker than that of the strain WS isolated in Londen in 1933. The antigenic structure differs distinctly from that of strain WS. The sera of 3 patients showed a distinct increase of their content in immune bodies against the strain WS and the isolated strain.

J. MULDER, *Haemophilus influenzae* and influenza-ultravirus met betrekking tot de etterige bronchitis. (*Haemophilus influenzae* and influenza ultravirus in connection with purulent bronchitis). Ned. T. voor Geneeskunde **84**, 2806, 1940.

Haemophilus influenzae of the PFEIFFER type is found in the greater part of cases of „ordinary" and (non-foetid) chronic purulent bronchitis (bronchiectasis), often as the numerically prevailing organism. The pathogenic properties of the PFEIFFER type in the bronchi are not yet strictly proved, but it can be considered as very probable that this group causes acute and chronic purulent bronchitis. It is improbable that ordinary purulent bronchitis and bronchiolitis are primarily caused by a still unknown brochiotropic ultravirus, and the infection with the *Haemophilus* group should be of a secondary nature only.

L. BIJLMER, Aetiologie der influenza. De isoleering van het influenza-virus tijdens de epidemie van 1941 te Groningen. (Etiology of Influenza. The isolation of the influenza virus during the epidemic of 1941 at Groningen). Diss. Groningen 1943.

The experimental studies on influenza, during the epidemic of January-February 1941 at Groningen (Holland), were carried out according to the technique evolved by WILSON SMITH, ANDREWES and LAIDLAW, of the National Institute for Medical Research at Hampstead (London). The investigations were carried out in a laboratory specially built in 1940 for the study of experimental influenza in ferrets and since 1941 incorporated in the Institute for Hygiene and Bacteriology of the State University.

During the period of January 9th to February 7th, material from 13 patients, partly throat-washings, partly suspensions of sputa, obtained during the first days of illness, was inoculated in ferrets, which were kept under rigid measures of isolation. The temperature was read twice daily and the clinical symptoms were recorded

In 5 cases the ferrets developed a typical influenza infection, with the characteristic fever peak on the second or third day and catarrhal symptoms of the nose, sometimes of the conjunctiva. In all these cases influenza antibodies against the virus strain WS could be demonstrated in the ferret convalescent serum by means of the mouse protection test.

In two other cases the signs in the ferrets were doubtful, but the ferrets' convalescent serum contained antibodies against the influenza virus, so that these animals must be assumed to have gone through a subclinical infection.

In 4 of the 6 remaining cases in which the ferrets did not show any reaction, the patients' convalescent serum neither showed any rise in antibodies In the remaining 2 both the mouse protection and complement fixation test of the patients' sera gave positive results, so that missed ferret inoculation must be assumed

Three virus strains were isolated, from three of the enumerated cases. After some ferret passages (3, 4 and 5) the virus was adapted to mice. The strains produced lethal pulmonary lesions in the mice after 9, 3 and 7 passages respectively.

In the course of the ferret and mouse passages the virus suspension was filtered through a collodium membrane (average pore diameter 0.6 μ). The throat-washings and sputa from the patients were not filtered before being inoculated in the ferrets, in order not to reduce the virulence of the pathogenic agent.

The nature of the pulmonary lesions of the mice was checked microscopically by sectioning the lung. Hereby the criteria described by STRAUB were accepted.

The analysis of the three isolated influenza virus strains did not show any mutual antigenic differences. The new strains were not found to be identical with any of the English „specific" strains.

The immunological study of the sera from fifty influenza patients demonstrated, that in 43 cases the influenza virus had been the etiological agent; among these were 13 cases of pneumonia, following an attack of influenza.

Prophylaxy and therapy by means of vaccine and immune serum are discussed. A multivalent anti-influenza serum was prepared by hyperimmunising rabbits with various virus strains. Some prophylactic effect of rabbit immune serum, intranasally administered to mice infected with influenza virus, could be noted.

The aim of this study may not be considered reached by the ascertainment of the etiology in a single influenza epidemic. This work must be seen as a preparation for the study of an influenza pandemic.

M. E. Kulsdom, Over de epidemiologie van meningococcosis en poliomyelitis anterior acuta. (On the epidemiology of meningococcosis and poliomyelitis anterior acuta). Ned. T. voor Geneeskunde **84**, 398, 1940.

The study of the occurrence of meningococcal infection makes it probable that clinical cases of this infection are always independent casualties, in which the germs have succeeded in overpowering an organism lacking in its normal means of defence. This fact and our knowledge of the distribution of meningococci among human individuals also gives rise to the assumption that biologically the symbiosis of man and meningococcus may be characterized as commensalism. A study of statistics and literature of acute anterior poliomyelitis furnishes data in support of the view that for the virus of this disease similar considerations are valid as for the meningococcus.

Ch. G. J. Dornickx en H. Peeters, Nekkramp bij militairen. (Epidemic meningitis among military men). Ned. T. voor Geneeskunde **84**, 4155, 1940.

During the mobilisation of 1939 32 cases of epidemic meningitis have come to knowledge in the mobilised Dutch armies. The opinion displayed in Holland by Bijl, Peeters and Siestrop, that epidemic meningitis is an illness preferably attaining soldiers and more especially recruits, is sustained by the collected data. For the control of the disease the testing of smears from contact-persons for the presence of meningococci is of no use. The great importance of sulfapyridine for the treatment of the disease is pointed out.

J. J. Th. Vos, Encephalitis toxoplasmotica. Ned. T. voor Geneeskunde **85**, 2401, 1941.

The section of a 5 weeks old child, which had suffered from indistinct cerebral symptoms showed a chronic necrotising inflammation in several parts of the brain. Some of the lesions showed signs of calcification, which made it probable that the disease had set in before birth. At first the cause of the encephalitis was unknown, but after the publication of cases of toxoplasmotic encephalitis by Wolf and Cowen the author re-examined the sections and was able to demonstrate the toxoplasma in several foci. Some were lying in so-called cysts, others free. A similar case has been published by C. C. de Lange. Here a child 3.5 months old was concerned, the disease of which initially was described as a case of hydrocephalus. Later the disease was identified by Wolf and Cowen as a toxoplasma meningo-encephalitis.

J. D. Verlinde en F. Wemsinck, Herpesvirus in den liquor cerebrospinalis van een lijder aan sclerosis multiplex. (Herpes virus in the liquor cerebrospinalis of a sufferer from sclerosis multiplex). Ned. T. voor Geneeskunde **86**, 3209, 1942.

A virus taken from the cerebrospinal fluid of a patient suffering from disseminated sclerosis has been isolated. This was inoculated in rabbits and from cross immunisation experiments it proved to be the virus of herpes. The patient suffered at the same time from repeated herpes and must be regarded as a virus carrier. The blood serum contained a large amount of neutralizing antibodies. In animal experiments the strain proved to be strongly antigenic.

E. F. J. H. Falger, Meningo-encephalitis na rubeolae. (Meningo-encephalitis after rubeolae). Ned. T. voor Geneeskunde **87**, 109, 1943.

Description of a fatal case of meningo-encephalitis after rubeolae in a woman of 26. In general the neurological complications after rubeolae begin very acutely. Practically without exception lymphocytes occur in the cerebrospinal fluid, which remains sterile. The anatomical changes in the central nervous system are probably caused by an anaphylactic reaction on the rubeolae-toxallergene.

A. C. Drogendijk, Bijdrage tot de casuïstiek van het zoster-varicellen vraagstuk. (Contribution to the casuistics of the zoster-varicellae problem). Ned. T. voor Geneeskunde **84**, 2931, 1940.

Description of two cases of herpes zoster followed by varicellae in persons who had been in contact with the patient.

A. C. Drogendijk, Over het aetiologisch verband tusschen zoster en Varicellae. (On the aetiological relation between zoster and varicellae). Geneesk. Bladen uit Kliniek en Laboratorium **37**, 325, 1940.

The author discusses the possibility of a close relation between the zoster and varicella virus with reference to literature study and his own observations

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J. D. Verlinde, De invloed van virulentie en herkomst van het vaccinevirus op het ontstaan van experimenteele encephalitis. (The influence of virulence and origin of the vaccine virus on the causing of experimental encephalitis). Ned. T. voor Geneeskunde **84**, 214, 1940.

Encephalitis can be caused by inoculating dogs cutaneously with vaccinia virus during a latent guanidine-intoxication. The

virulence of three different strains of dermovaccine appeared to have no influence on the causing of experimental encephalitis. Neurovaccine was stronger encephalitogenic than dermovaccine. In herbivorous animals encephalitis could not be induced in this way, not even in calves after vaccination with the original cowpox virus.

J. D. Verlinde, Over de pathogenese van de encephalitis post-vaccinalis. (On the pathogenesis of the encephalitis postvaccinalis). Maandschr. voor Kindergeneeskunde **9**, 368, 1940.

It is possible to induce a post-infectious encephalitis in the course of dog-distemper by inoculating dogs with the distemper virus during a guanidine-intoxication. The same result is obtained in dogs with the distemper virus by vaccinating them against smallpox during a guanidine-intoxication. With rabbits, calves and monkeys experiments did not succeed. Probably in these animals and perhaps also in men, another form of auto-intoxication from the intestinal tract plays a role in the etiology of post-vaccinal encephalitis. Several strains of dermovaccine, but especially neurovaccine were found to have encephalitogenic properties. Preventive and curative measures should be directed against predisposing metabolic factors.

B. H. Wormgoor, Koepokken (Variola bovine). (Cow pox. Variola bovina). Ned. T. voor Geneeskunde **86**, 2185, 1942.

The development of vaccine pustules is described in two dairymen who probably had transmitted the virus themselves from a vaccinated infant upon the cattle.

G. F. Bohré, De luesreactie van Chediak. (The syphilis reaction of Chediak). Ned. T. voor Geneeskunde **86**, 1279, 1942.

The technic of the micro-syphilis reaction of Chediak is described. After mentioning the good results of extensive experiments elswhere, the results of personal investigations are given. In concluding, attention is called to the various advantages of the test, a.o. its special suitability for group testings for syphilis.

J. H. Bekker, De waarde van de reactie volgens Chediak voor de serologische syphilis-diagnostiek. (The value of the reaction of Chediak for the serological diagnostic of syphilis). Ned. T. voor Geneeskunde **86**, 1268, 1942.

The reaction of Chediak is discussed, a serologic syphilis reaction carried out with one drop of dried blood. On examination of 473

blooddrops, against the WASSERMANN reaction a gain of 2 % and a loss of 1 % were obtained, whereas in 1.5 % of the cases a non-specific reaction was seen. The reaction of CHEDIAK, therefore, is quite practicable for the detection of syphilis in cases where difficulties are encountered in carrying out the other reactions, if only one keeps in mind that in case of a positive reading it remains necessary to submit the blood to a complete serologic examination.

ANIMAL PATHOLOGY

J. D. VERLINDE, Aetiologische, epidemiologische en serologische onderzoekingen over adenitis infectiosa equorum. (Aetiological, epidemiological and serological investigations of adenitis infectiosa equorum). Tijdschrift voor Diergeneeskunde **67**, 636, 1940.

Studying strangles of army horses two forms were met with, *viz.*, one in which an uncomplicated catarrh of the larynx and trachea and one in which abscesses of the retropharyngeal and submaxillar glands dominated. In either form streptococci could be isolated from the purulant discharge of the nose or the glands. Two out of eight strains of the streptococci diverged somewhat in their fermentation of lactose. The cultures were pathogenic for mice and rabbits. Culturally identical strains appeared to differ widely in agglutination tests. The atypical strains were strongly agglutinated by all examined sera of convalescent horses, the typical strains only in small measure and then hardly by other homologous serum.

J. D. VERLINDE, Encephalomyelitis equi. Maandschr. voor Kindergeneeskunde **8**, 181, 1940.

Since 1938 many cases of encephalitis in man have been observed, caused by the virus of horse encephalitis which in this continent occurs epizootically in horses. Although people of all ages are sensitive for the virus, most of the cases are found in children. This disease, unknown in our country, is discussed in a general survey in which its importance for men is pointed out.

JAC. JANSEN, Goedaardige droes, adenitis equorum. (Mild strangles, adenitis equorum). Tijdschrift voor Diergeneeskunde **67**, 141, 1940.

Streptococcus equi was isolated directly in pure culture from 12 typical and 1 atypical case. *Str. pyogenes* (EDWARDS' type A) was isolated directly in pure culture, from pleura exudata of a horse, also, but not directly in pure culture from a gland of an atypical

mild case, resembling strangles, five times from the discharge of the nose and once from abscesses caused by serum injections. Two identical streptococci, which colored bloodagar slightly green, were cultivated, one from the kidney and the spleen and one from a bursted gland. The major differences between the cultures of *Str. equi*, *Str. pyogenes* and *Str. viridans* were:

strains	hemo-lysis	pro-ducing green colour	sorbitol	lactose	trehalose	litmus milk
Str. equi	+	—	—	—	—	pale
Str. pyogenes	+	—	+	+	—	acid, clot
Str. viridans	—	+	—	—	+	pale, some acid

Some strains cultivated from the throats of persons, suspected of being infected with strangles, did not agree with the above strains.

Jac. Jansen, *Shigella equuli* (*B. pyosepticus equi*)-infecties. (Infection by *Shigella equuli* (*B. pyosepticus equi*). Tijdschrift voor Diergeneeskunde **68**, 687, 1941.

Shigella equuli does not only occur in foals and pigs, but also in full-grown animals. The author diagnosed the disease in an 1½ year old horse which had died from acute septicaemia. From the pus of a case of chronic funiculitis in a 9 years old horse, *Shigella equuli* was isolated. It has also been found in the organs of a pig, which had died from acute septicaemia.

L. W. Janssen, Het mond- en klauwzeervraagstuk bekeken van de chemische zijde. (The problem of the foot and mouth disease looked at from the chemical side). Tijdschrift voor Diergeneeskunde **67**, 10, 1940.

The author gives a summary of his conception of the nature of the virus of foot and mouth disease and of the disease itself. Starting from the size of the virus, the resistance against chemicals differing from bacteria, the inactivation by pH between 4,0 and 6,5, the insensibility for bacterial substrates, the lacking of metabolism, the predilection spots and the connection between the virus and the cells in which it is formed, the author had reached the conclusion, that this virus must be a dead protein.

In his own experiments, the influence of chemical and physical factors was established. The virus was resistant to the action of alcohol and ether at a low temperature. It behaved as a dead

protein and not as a living protoplasm, that is disturbed by the lipoid extraction. Toluol, chloroform and ether are rather good bacterial poisons, but they interfere but little with enzymatic reactions. They are good conservatives for the virus. The virus behaves as a protein or enzyme also when shaken with these solvents. The inactivity of ferment poisons (KCN) which completely stop the respiration of living organisms in a much smaller concentration, is put forward as a sufficient proof of the dead nature of the virus.

In different ways, recorded before, it was possible to obtain a protein fraction containing the virus in a very pure form. One gram of this is sufficient to infect a milliard guinea-pigs. Examination of the chemical structure of the protein, considered as virus made it likely that it is a nucleoproteid containing ribose. The observed increase of the virus is not due to division of an independant micro-organism, but the virus is a pathological product of the plasm of the epithelial cells of the skin. These cells are not host cells, but they have a disturbed metabolism. The normal epithelial cells produce keratin fibrills, the sick cells produce virus instead of keratin. This conception agrees with the histological findings.

L. de Blieck en Jac. Jansen, Enting tegen mond- en klauwzeer met crystalviolet-vaccin alsmede met bij 37° C. gedood virus bij de cavia. (Inoculation against foot- and mouth disease with crystal violet vaccine and with virus killed at 37° C. in the guinea-pig). Tijdschrift voor Diergeneeskunde **69**, 47, 1942.

Immunity against foot- and mouth disease could be induced in the guinea-pig by inoculation with virulent blood, kept for 10 days at 37° C. The same result was attained by adding crystal violet. For both methods the results were better with a filtrate of epithelion than with blood. A single subcutaneous injection sufficed for the immunization of the guinea-pigs. After vaccination a humoral immunity is formed, setting in after 3 days and lasting for at least 52 days. A further injection in the humoral immune guinea-pig causes a local reaction and complete immunity.

A. F. van der Scheer, Eenige waarnemingen over in Nederland voorkomende mastitisstreptococcen bij het rund. (Some observations on in the Netherlands occurring mastitis streptococci in cattle). Tijdschrift voor Diergeneeskunde **67**, 76, 1940.

A. F. van der Scheer, Over mastitis veroorzakende streptococcen. (On Streptococci causing mastitis). Thesis, Wageningen, 1941.

The biochemical and serological characteristics of the streptococci causing mastitis in cattle are described. The new name of

Streptococcus pyosepticus is suggested for the haemolytic group of C-streptococci.

The detection of the haemolytic property was greatly simplified by the use of stab-cultures in blood-agar-slants. For the serological differentiation the precipitin test of LANCEFIELD was used. Sera were prepared in the usual way. For the preparation of extracts a method was worked out.

The examination of 312 strains of *Str. agalactiae* showed that the slimy consistence of the colonies of 80 to 90% of the strains on horse-serum-agar is of great importance for a quick recognition of the species. Moreover about 60 % of the strains with non-slimy colonies can be recognised by the colonies „in shape of wound thread". Nearly 50 % of the strains induce haemolysis. The area of haemolysis is usually small, but about 10 % of the haemolytic strains produce very large areas. 77 % of the streptococcal mastitis in cows are caused by *Str. agalactiae.*

The examination of 175 strains of *Str. dysgalactiae* showed that the colonies are never slimy or in „shape of wound thread". On horse-serum-agar about 95 % of the strains cause a turbidity in the agar under each colony. After removal of the colonies from the surface of the agar this phenomenon is very conspicuous. It was shown that some 30 % of the strains show a slow production of acid from salicin and some 20 % cause a weak hydrolysis of sodium hippurate. By means of the precipitin-test and reciprocal absorption-tests it was proved that *Str. dysgalactiae* belongs to the serological group C and that the deviating strains just mentioned should in fact be regarded as *Str. dysgalactiae.* 14 % of the number of cases of streptococcal mastitis was caused by *Str. dysgalactiae.*

83 strains of *Str. uberis* were examined. The surface colonies on horse-serum-agar often show a close resemblence to those of *Str. agalactiae.* Even slimy colonies may occur. A few strains may cause a turbidity in the agar like *Str. dysgalactiae.* It was demonstrated that some 30 % of the strains do not form acid from inulin. Moreover the hydrolysis of sodium hippurate is extremely weak in several cases, so that the result may easily be recorded as negative. By means of the precipitin-test and the absorption-tests it is proved that these deviating strains should indeed be regarded as *Str. uberis.* More than 60 % of the strains contained group specific precipitinogens. Group specific sera are only obtained from strains containing no type-specific precipitinogens. The serological group is not indicated by a letter, because it is considered likely that *Str. uberis* will prove to belong to group E. This supposition however cannot be substantiated, because a comparison with American sera was impossible. The number of cases caused by the species was 7 %.

68 strains of *Str. pyosepticus* offered data in complete agreement with those presented by other authors. Acid is formed from sorbitol but not from trehalose. The precipitin-test with group C serum is always positive. In cows this species caused 2 % of the

cases of mastitis. In other animals especially in horses *Str. pyosepticus* occurs more or less frequently in various infections.

Str. pyosepticus var. *humanus* was isolated from cow-milk only once, but not in connection with mastitis. Acid is formed from trehalose, but not from sorbitol. Group C serum is precipitated.

Str. pyogenes could not be isolated from cow milk. Biochemical tests cannot serve as means of a definite differentiation of these strains from *Str. pyosepticus* var. *humanus*. A group specific serum prepared from *Str. pyogenes* is considered as a group A serum.

Horse-serum-agar is an excellent medium for growth and isolation of mastitis streptococci. By the addition of 0.05 to 0.1 % aesculin this agar becomes excellently suited for diagnostical purposes. The hydrolysis of aesculin is easily detected under a quartz lamp. When only non-aesculin splitting strains are considered, the characteristics for the colonies of *Str. agalactiae* and *Str. dysgalactiae* become specific. *Str. uberis* and other streptococci, with colonies showing the same characteristics, all decompose aesculine.

L. F. D. E. Lourens en A. F. van der Scheer, Over het verloop en de genezingskans van de mastitis door *Streptococcus dysgalactiae* en *Streptococcus pyogenes*. (On the course and the chance of recovery of mastitis by *Streptococcus dysgalactiae* and *Streptococcus pyogenes*). Tijdschrift voor Diergeneeskunde **68**, 283, 1941.

A mastitis caused by *Str. dysgalactiae* is acute. A latent infection however of the udder may occur. The thus induced mastitis can nearly always be cured by repeated milking, preferably combined with an auto-vaccin treatment. It rather often occurs in connection with other disturbances. The danger of spreading the infection after the mastitis has appeared is rather small.

The mastitis caused by *Str. pyogenes* was severely acute in more than half of the number of cases examined. In the other cases the mastitis was chronic, without acute initial stage. The quarters affected by *Str. pyogenes* hardly without exception must be given up for lost. The after-effects of the mastitis (emaciation) may possibly be combated by opening of the teats. The infection by *Str. pyogenes* spreads easily to other quarters of the same cow. Hygienic measures to prevent a possible spread of the infection to other cows are advisable. In exceptional cases the mastitis by *Str. pyogenes* may occur during the period of refreshing.

W. van den Berg, Een nieuwe methode ter onderkenning van streptococcen en staphylococcen in de melk van klinisch normale uiers. (A new method for the distinction of Streptococci and Staphylococci in the milk of clinically normal udders). Tijdschrift voor Diergeneeskunde **67**, 8, 1940.

Under aseptic precautions 1—2 ml milk is brought straight from

the teats into a sloped tube with serum agar. After incubating for 18 hours the result can already be noted.

J. VAN DER HOEDEN, Komt *Brucellosis suis* in Nederland voor? (Does *Brucellosis suis* occur in the Netherlands?). Tijdschrift voor Diergeneeskunde **67,** 226, 1940.

Among 87 *Brucella* strains isolated by the author from men and animals not one of the suis type could be detected. Serological tests of 486 slaughter swine in merely 16 cases gave an agglutination against *Brucella* but the titers were of no conclusive value. It is possible that those feeble reactions have been caused by a symtomless infection with *Brucella Bang,* originating from cattle. Up till now *Brucellosis suis* is not met with in the Netherlands.

C. J. DE GIER, Is de „Teschener Krankheit" (Schweinelähme. Encephalomyelitis) een afzonderlijke ziekte of wordt zij veroorzaakt door het virus van de varkenspest? (Is the „Teschener disease" (Schweinelähme, Encephalomyelitis) a separate disease or is it caused by the virus of the swine-plague?). Tijdschrift voor Diergeneeskunde **68,** 695, 723, 1941.

The author poses this question in view of the occurrence of swine-plague with obvious paralytic symptoms, whilst no macroscopically visible pathological changes are to be found, but merely perivascular infiltration and morbid growth of glia in the central nervous system. As the diagnosis of the Teschener disease is based on these symptoms it is not out of the question that both diseases are identical. Paralytic symptoms also accompany other diseases of pigs (Aujeszky disease, salt proteid intoxication, vitamine A deficiency) and so do perivascular infiltrations and morbid growth of glia (bronchopneumonia, swine influenza, swine pox, paratyphoid, Aujeszky disease, worms and excessive feeding with proteins). This is of significance, as such pigs might be used as test animals for the Teschener disease.

H. H. VINK, Mucormycose bij een varken. (Mucor mycosis in a pig). Tijdschrift voor Diergeneeskunde **68,** 312, 1941.

The author mentions a case of mucor mycosis, caused by *Absidia Lichtheimi* in a pig. The progress was localized in both submaxillar glands and the left inguinal gland. Histologically a fairly strong eosinophily is recorded.

A. Bauvéry-Asman, De beteekenis der seroreactie bij honden met betrekking tot leptospiren infecties. (The signification of the seroreaction in dogs in connection with the infection by Leptospira). Tijdschrift voor Diergeneeskunde **67,** 799, 1940.

In 280 dogs, taken at random, the agglutination-lysis test against leptospira has been carried out. About 40 % were ascertained as positive. These dogs were divided in those with a low and those with a high titer. In the latter the rate of *Leptospira icterohaemorrhagiae* to *Leptospira canicola* was 1 : 2, a same rate as has been found in clinically sick animals. The low titer might perhaps be caused by a slow repeated immunization.

In about 50 cats the seroreaction was negative. This does not exclude the possibility of an infection, although not making it probable.

The progress of the titer of dogs which had suffered from leptospirosis has been followed. The increase is rather quick, after 4,5—7 weeks the maximum is reached, then a decrease follows, but much slower. A titer may remain for years. This is of value for the serological test. Only a repeated examination of the blood can tell us something about the patient.

Jac. Jansen, Over *Clostridium Welchii*-infecties. (On infections by *Clostridium Welchii*). Tijdschrift voor Diergeneeskunde **68,** 562, 1941

From a phlegmon in the wall of the stomach of a dog *Cl. Welchii* was isolated in pure culture and also from the urine of a living dog and from the bladder of the same dog after its death. The surroundings of the bladder and the prostate were phlegmonous. The latter strain digests glycerol but not inulin.

Jac. Jansen, Konijnenpest. (Rabbit's plague). Tijdschrift voor Diergeneeskunde **68,** 967, 1941.

In rabbits a filterable virus was detected which was very pathogenic when inoculated subcutaneously or intranasally. Contact infection was easily proved. The virus has been cultivated on the egg membrane.

Jac. Jansen, Experimenteel onderzoek van konijnensterfte door een filtreerbaar virus. (Experimental investigation of mortality in rabbits caused by a filterable virus). Tijdschrift voor Diergeneeskunde **69,** 504, 1942.

A spontaneous acute disease with high mortality (rabbit plague) was observed in rabbits. A filterable virus proved to be the cause. The virus was ascertained in blood, urine, gall and mucus of the

nose. It is possible to transmit the disease to rabbits by subcutaneous, intradermal, intranasal, conjunctival, intravenous inoculation and by infection per os and by contact.

M. F. Polak, Épidémie survenue parmi des souris blanches à la suite d'une infection par le *Corynebacterium pseudotuberculosis murium*. (Epidemic set in among white mice as the result of an infection with *Corynebacterium pseudotuberculosis murium*). Antonie van Leeuwenhoek **10**, 23, 1944—1945.

An epidemic attaining white laboratory mice has presented the clinical picture of pseutuberculosis of the liver. The cultures of the isolated organism have revealed *Corynebacterium pseudotuberculosis murium* which for various reasons is considered as the causal agent. This micro-organism has merely provoked the pneumonia with foci regularly described in literature when it has been administered per os with the food.

H. Vervoort and A. Charlotte Ruys, The recognition of Psittacosis. Antonie van Leeuwenhoek **6**, 11, 1939—1940.

The psittacosis virus seems to be endemic amongst cage birds in Amsterdam. In animal experiments it is of low virulence. It gives only occasionally rise to isolated human cases or small outbreaks. The results of animal experiments and complement-fixation tests with material from human cases and infected birds are recorded. A description is given of a reliable staining method for the virus. The value of the complement-fixation test is discussed.

L. de Blieck, Immunisatie tegen *Coryza infectiosa gallinarum*. (Immunization against *Coryza infectiosa gallinarum*). Tijdschrift voor Diergeneeskunde **69**, 204, 1942.

By intravenous injection of living or formalized cultures of *Haemophilus coryzae* de Blieck fowls can be immunized against coryza caused by that organism. Against coryza caused by the so-called Nelson bodies complete immunity can be induced by intravenous, intramuscular and intrabursal (bursa Fabricii) injection of exudate from the cella infraorbitalis. Intravenous immunization gives the best results.

A Bos, Weer nieuwe gevallen van eendenpest. (New cases of duck plague). Tijdschrift voor Diergeneeskunde **69**, 372, 1942.

New cases of duck plague were diagnosed. It caused an important mortality in some large duckfarms neighbouring each other. On

5 farms in the course of some months 2600 of 5700 animals died. Other animals fell ill but recovered and appeared immune afterwards. The symptoms were lameness, soon followed by paralysis (especially of the legs, creeping locomotion), further violent thirst, sometimes stinking discharge from the bill, diarrhoea with greenish and yellow coloured sloppy stools. Incubation period together with period or progress of the disease takes about a week. Dissection: petichia of the heart and sometimes also of the other internal organs and in the trachea. Fibrinous or fibrinopurulent peritonitis cophoritis with large haemorrhages in the egg follicles.

The virus could be kept on in a series of 18 passages through ducks in the course of more than a year. Intramuscular injection of heart's blood conserved in 50 % of glycerol preserved at 5° C. suffises for the keeping on of the virus.

Ducks wich had remained negative after inoculation appeared to be immune to superinfections, so did a recovered duck from one of the infected farms. No infection could be provoked in fowls, pigeons, rabbits, guinea-pigs, rats and mice.

The causative agent of the ducks plague is assumed to be a separate kind of virus and not a variety of fowl plague virus.

C. J. DE GIER, Het cultiveeren van *Trichomonas foetus*. (The culturing of *Trichomonas foetus*). Tijdschrift voor Diergeneeskunde **67**, 902, 1940.

The medium for the culturing of *Trichomonas foetus* such as it has been recommended by SCHOOP and OEHLKERS is preferable to those which contain more protein. The liquid from the allantois also is very suitable as a culture medium and quickly leads to success. Scrapings of cotyledones mixed with 0.9 % saline have no value as a culture medium.

A. Bos, Die Trichomoniasis der Tauben und ihre Bekämpfung. (The trichamoniasis of pigeons and its combating). Thesis, Utrecht 1941.

Trichomoniasis in Holland is one of the most widespread infectious diseases of pigeons. To obtain a pure culture TAROZZI-broth is most suitable. In this medium a pure culture of *Trichomonas hepatica* can be cultured contineously for 1 year and a half in 162 generations. The egg-medium of LOCKE is very well suited to obtain a pure culture from material contaminated with bacteria. In blood broth and blood milk the parasite multiplies as well. Culturing on a solid medium had no success.

Jac. Jansen, Pokken bij de kauw. (Pox in a tame jackdaw). Tijdschrift voor Diergeneeskunde **69**, 128, 1942.

Spontaneous pox was observed in a tame jackdaw (*Colaeus monedula*). The virus was ascertained as the canary-pox virus.

TECHNICAL MICROBIOLOGY

T. Folpmers, New enrichment methods for the cultivation of *Bacterium coli* and faecal Streptococci in water samples. Antonie van Leeuwenhoek **6**, 22, 1939—1940.

The results of a series of tests extending over a period of three years in the Rotterdam water works are reported. For the detection of *Bacterium coli* very satisfactory results were obtained with:

1) a medium containing 1 % glucose, 0.5 % glutamic acid neutralised by NaOH, 0.5 % ammoniumlactate, 0.5 % NaCl, 0.3 % K_2HPO_4 and tapwater (pH = 7.0). Cultivation at 45° C. in completely filled stoppered bottles.

2) a medium containing 0.3 % bacto-tryptone, 0,1 % sodium formate, 0.04 % K_2HPO_4 and 0.0001 % crystal violet, dissolved in destilled water (pH = 6). Cultivation is performed at 45° C. in completely filled stoppered bottles. After 24 and 48 hours gas production is observed and a test for indole formation is made. As a rule they coincide.

For the selective enrichment of *Streptococcus faecalis* best results were obtained with:

1) a medium containing 1 % peptone (Poulenc, Difco or Bacto), 1 % lactose, 0.5 % NaCl (pH = 7.0). Litmus added up to a deep purple colour. Cultivation in test-tubes filled to a depth of 10 cm or in Erlenmeyer flasks at 45° C.

2) a medium containing 1 % peptone (Poulenc, Difco or Bacto), 1 % caffein, 0.1 % glucose, 0.3 % Liebig's beef extract, 0.5 % NaCl (pH = 7.2). Incubation at 37° C. in completely filled stoppered bottles.

T. Folpmers, An improvement in the bacto-tryptone, sodium formate medium for the detection of *B. coli*. Antonie van Leeuwenhoek **10**, 28, 1944—1945.

An improvement of the bacto-tryptone, sodium formate medium (cf. Antonie van Leeuwenhoek **6**, 22, 1939—1940) which has been put on trial for nearly a year in the Laboratory of the Rotterdam Waterworks, consists in lowering the content of crystal violet to half of the original amount and raising the pH to 6.8.

T. Folpmers, On the disappearence of *B. coli* and faecal Streptococci (Enterococci) as the result of slow sand filtration. Antonie van Leeuwenhoek **7**, 104, 1941.

During three successive years counts were made of *Streptococcus faecalis* and *B. coli* in the prefiltered water (primary filtrate) and the slow sand filtered water (secondary filtrate) of the Rotterdam Waterworks. The faecal streptococci merely appeared in the secondary filtrate in the winter months and in highest numbers when the temperature of the water under the ice deck sinks below 1° C. In summer not any streptococci can be detected in samples of 100 ml of the primary filtrate. The number of *B. coli* increased as well at low temperatures. In secondary filtrate streptococci appear later in the year than *B. coli* and as soon as the ice in the filters is melted they disappear at a much earlier date than *B. coli*. Protozoa which are the natural enemies of streptococci encyst as a result of low temperature and this entails the increase of streptococci in winter.

The bacteriological situation especially in winter may be dangerous and properly applied and bacteriologically controlled chlorination will be essential.

P. C. Flu, Are bacteriophages significant for the self-purification of surface water? Antonie van Leeuwenhoek **7**, 39, 1941; Cf. also: Acta Leidensia **15—16**, 63, 1940—1941.

As early as 1926 the author has claimed that in the self-purification of water (under experimental conditions realized in the laboratory) bacteriophages take no part, at least not an easily discernable one. The investigation of Schuurman on the significance of typhoid phages in the self-purification of the water of the Tjiliwoeng river (Netherlands East-Indies) induced the author to attack the problem once more from the experimental side. The results of these recent investigations allow no other conclusion than that under circumstances and conditions as have been fully described, bacteriophages do not act on the self-purification of water. It might be conceived that the high temperature of the Tjiliwoeng river and its high content of colloidial clay allow the phage to play a certain part, although the results of the author's experiments make this hardly probable. At all events such an influence is neither proved nor made probable by the results of Schuurman's experiments with the untreated Tjiliwoeng water, because of his failing to exclude the action of the protozoa.

R. Abdoelrachman, Can we use the Shiga-bacteriophage as indicator for a faecal pollution of water? Antonie van Leeuwenhoek **9**, 143, 1943; Cf. also: Wateronderzoek met een bacteriophaag-

methode. (Water control by means of a bacterophage method). Thesis, Utrecht 1942.

In an important percentage of stools of healthy persons Shiga bacteriophage occurs. The Shiga bacteriophage occurs during some lapse of time in water which has been polluted by human faeces. The Shiga bacteriophage could be ascertained in water by means of the enrichment method of NYBERG. The Shiga bacteriophage method for indicating the pollution of strongly polluted water is as sensitive or sometimes less so than the methods of EIJKMAN and of CLEMESHA. For the examination of water in several stages of purification in water works the Shiga bacteriophage method is as sensitive or even more so than the other methods. The Shiga bacteriophage does not act strongly in the self-purification of polluted water. It may be used as indicator for a faecal pollution of water and this method may be applied along with the other bacteriological methods in water control. It may be advantageous to study more closely its limits and its benefits in the practice of water control.

H. DE GRAAF, A revision of the procedure for the Voges-Proskauer test. Antonie van Leeuwenhoek **7**, 92, 1941.

The recommended method is the following: The medium consists of 2 % glucose, 0.1 % K_2HPO_4, 0.05 % $MgSO_4$, 0.1 % NH_4Cl (or $(NH_4)_2SO_4$) and 1 % $CaCO_3$. The culture is incubated for 24 hours (or if necessary for 48 hours) at 37° C. To three drops of the culture solution in a test tube three drops of a 4 n NaOH solution and one drop of a 1 % creatine solution are added. The mixture is placed in a waterbath at 45—50° C. and is shaken now and then. In case of a positive result the red colour will show in one or two minutes.

JAN SMIT, B. M. KROL and A. J. VAN WIJK, The *B. coli* test in the routine analysis of raw milk. Antonie van Leeuwenhoek **6**, 1, 1939—1940.

It is emphasized, that the estimation of coliform bacteria is important in the routine analysis of raw milk. Various tests recommended in the literature are described and stress is laid on the fact, that only those which may be completed within 24 hours are of actual value. A prescription is given of a satisfactory test using a broth of nearly the same composition as the well known Endo agar, and a comparison is made of the results of this test with 3 other tests in investigating 30 samples of raw milk. It is proved that a considerable number of the coliforms found are true *B. coli* which have lost the power of indole formation.

N. Savrij, Eenige vergelijkende onderzoekingen over het aantoonen van coli-bacteriën in gepasteuriseerde melk. (Some comparitive investigations of the means of detecting *B. coli* in pasteurised milk). Chem. Weekblad **38,** 114, 1941. Officieel orgaan van den Algemeenen Nederlandschen Zuivelbond **36,** 55, 1941.

Compared are: The method of the milk decree 1916 (enrichment in acid broth), the method of an official control instance (enrichment in meat broth with lactose and neutral red and cultures on Endo plates), and the method of the laboratory of the Cooperative Plant of Milk products at Bedum (enrichment in a peptone-lactose-brillant green-gall medium and culture on eosine-methylene blue plates.

The results vary, especially when the fermentation test is negative. It became evident that on the Endo plate red colonies even those with a metallic lustre are often not due to *B. coli*. On the other hand on the eosine-methylene blue plates colonies with a metallic lustre are always formed by bacteria of the coli group.

Therefore it seems desirable to base the research for *B. coli* in pasteurised milk on: 1. the occurrence of gas in a lactose containing medium. 2. the metallic lustre of colonies on eosine-methylene blue plates.

C. F. van Oyen, Kleine plaat methode. (Little plate method). Handelingen van het Genootschap tot bevordering van melkkunde **1940,** I, p. 3.

Van Oyen's modification of Frost's little plate method is recommended for the bacterial count in milk of good bacteriological quality. 0.1 ml milk is mixed with 0.1 ml broth-peptone agar and spread over a surface of 20 × 50 mm. Incubation at 28—30° C. for 20 hours. After drying the slides are stained with carbol-thionin and the agar decolorised in running water. The colonies are counted by means of the microscope (50 ×), under a cover glass of 20 × 50 mm divided in spaces of 1 mm^2, resting on the agar slide. This method is not suitable for pasteurised milk as the bacteria develop too slowly.

A. Pasveer, De toepassing van de rolcultuur bij het bacteriologisch onderzoek van melk en melkproducten. (The use of Julius' count method in the bacteriological control of milk and milk products). Officieel orgaan van den Algemeenen Nederlandschen Zuivelbond **35,** 473, 1940.

For the estimation of the bacterial count of milk Julius' method was applied (Antonie van Leeuwenhoek **5,** 28, 1938—39). In small cylindrical glass jars with a cover of aluminium 3 ml liquified gelatine or agar is inoculated with a small quantity of milk, after which the jars are placed in an apparatus in which they are revolving rapidly around their cylindrical axis to solidify the medium

in a thin layer on the cylindrical wall. After incubation the colonies are counted. Non-lactic acid bacteria in sour milk products are estimated on a medium containing no sugar, unto which 0.05 % $CaCO_3$ has been added to prevent a decrease in pH caused by the presence of lactose in the inoculum. The sour milk products have to be solved in four times their volume of a solution of 20 g sodium-hexametaphosphate and 22 ml n NaOH in 1000 ml of water. The counting of bacteria in butter was performed by means of this method, using the butter serum as inoculum. The method is also suitable for the counting of yeasts and molds, when an appropriate medium is used.

Jaarverslag van den Gezondheidsdienst voor vee in Friesland. (Annual Report of the sanitary service for cattle in Friesland). Officieel orgaan van den Algemeenen Nederlandschen Zuivelbond **36**, 535, 1941.

The value of the reductase test is discussed. After the tubes had been read for the reductase test the same tubes were judged after 12 hours incubation. It appeared that peptonisation and acidification are of no importance for the classing of the milk for making cheese, as the milk samples of the best bacterial quality may induce these changes. The gas formation did not correlate with the results of the coli test in fresh milk. Although the fermentation may be improved by addition of rennet, as the coagulum retains the gas bubbles, the test gives no reliable indications.

A. K. van Bever en J. Straub, De beteekenis van Storch-reactie, oproming en phosphatasebepaling voor het toezicht op de melkpasteurisatie. (The significance of the reaction of Storch, raising of the cream and estimation of phosphatase for the control of the pasteurisation of milk). Chemisch Weekblad **38**, 210, 1941.

The importance of the phosphatase test for the hygienic control of milk pasteurised during a few seconds at high temperature (Voltana and Stassano milk) is emphasised. The phosphatase test should be negative. As the temperature and time interval between the killing of the tubercle bacteria and a negative phosphatase test is but small, a reliable control is necessary and in the testing of a new apparatus the cavia test must not be eliminated.

Jaarverslag van den Gezondheidsdienst voor vee in Friesland. (Annual Report of the sanitary service for cattle in Friesland). Officieel orgaan van den Algemeenen Nederlandschen Zuivelbond **35**, 549, 1940.

The possibility of estimating the bacteriological quality of milk was studied. Of milk samples containing less than 50.000 bacteria

per ml according to the plate method (Difco agar 22° C.) 28 % showed a bacterial count of more than 480.000 in the Breed test. Out of milk samples in which 51.000—100.000 were found with the plate method 42 % had according to the Breed test to be classed as containing more than 480.000 bacteria. When the milk samples were divided according to the Breed test in class I with a bacterial count below 600.000 and class II with a count above 600.000, 12 % of the samples containing less than 50.000 bacteria according to the plate method had to be placed in class II. The Breed count is therefore unreliable. Best results are to be expected with 3 Breed classes, *viz.*, I below 240.000, II 240.000—480.000 and III above 480.000. When classed according to this schedule 72 % of the samples with a plate count of less than 50.000 belonged to class I and only 4 % to class III. With milk samples of 50.000—100.000 these numbers were 58 % and 5 %. The little plate test of FROST-VAN OYEN gave merely half the amount of bacteria found with the plate test.

With the reductase test nearly all samples with a plate count of less than 200.000 reduced in less than 4 hours, but so did a larg enumber of samples with a high bacterial count. WILSON's method (turning every hour) gave better results and 3 classes are recommended (less than 4 hours, 4—6 hours and more than 6 hours).

J. VAN BEYNUM en J. W. PETTE, Een methode voor het aantonen van boterzuurbacteriën, speciaal geschikt voor het onderzoek van melk. (A method for the detection of butyric acid bacteria in milk). Verslagen van landbouwkundige onderzoekingen **46**, 379, 1940 and Jaarverslag Proefzuivelboerderij over 1940, p. 9.

A method for the detection of butyric acid bacteria in milk is described. The fermentation test is carried out in V-shaped tubes, one end of which has a rubber stopper and the other is cotton plugged. To 50 ml of the milk to be tested are added 1 ml of a 25% dextrose solution to enable the development of *Cl. tyrobutyricum* and 1 ml of 0.1 n HCl to inhibit the growth of putrifactive Clostridia. 4 or 5 V-tubes are filled with 10 ml of this mixture and on the surface of the milk in the cotton plugged arm is poured 1 ml of liquid paraffine. After pasteurisation during 10 minutes at 80° C. the tubes are incubated at 35—40° C. The test is positive when gas formation takes place within two or three days and the liquid is smelling of butyric acid. The milk is deemed to be seriously contamined when all tubes are positive. The contamination is insignificant when only one tube out of 4 or 5 is positive. The method is also suitable for the detection of butyric acid in other than dairy substances. Sterilised milk has to be used then.

J. van Beynum en J. W. Pette, De invloed van de voeding van het vee op de besmetting der melk met boterzuurbacteriën. (The influence of the feed of the cows on the contamination of milk with butyric acid bacteria). Verslagen van landbouwkundige onderzoekingen **46,** 397, 1940 and Jaarverslag Proefzuivelboerderij over **1940,** p. 27.

The milk of the experimental farm has been tested with the above method. When the cows were in the stable the milk was practically free from butyric acid bacteria, if they were fed on hay and artificially dried grass as a roughage. When a silage containing butyric acid is fed, the milk is strongly contaminated. There is also some contamination of the milk of cows in the same stable, not receiving silage. When the feeding of silage is brought to a close, it may last some 3 weeks before the milk is free from butyric acid bacteria. The milk of cows in the pasture generally does not contain butyric acid bacteria.

C. I. Kruisheer, P. C. den Herder, W. C. Smit en A. de Haan, Het bacteriologisch-chemisch kwaliteitsonderzoek der Nederlandsche keuringsboter. (Bacteriological and chemical grading tests of controlled butter in the Netherlands). 's-Gravenhage, Algemeene Landsdrukkerij 1940.

About 1000 butter samples derived from the Laboratory for control of dairy products have been studied as to the correspondence of organoleptic tests performed by experienced butter testers with the results arrived at by means of laboratory tests. All butter had been prepared from pasteurised cream and had been stored before ripening during 7 days at 13° C. Estimated were: the amount of catalase, total counts of microbes on casein agar, counts of moulds, yeasts, fat-splitting and protein-splitting micro-organisms, coliform bacteria, lactic acid bacteria on china blue-lactose-agar, and non-acidifying microbes. The chemical estimations bore on: visible water drops, NaCl, iron and copper compounds. The correlation between the score and estimations according to the laboratory tests has been calculated and although in the fresh butter samples no strong development of faults in odor or taste could occur, a correlation could be established between the score arrived at organoleptically and the amount of catalase, total count of micro-organisms on casein agar, count of molds, visible drops of water and the occurrence of the metals mentioned. The correlation was less close with the number of yeasts, of fat-splitting-, of protein-splitting- and of coliform bacteria. No correlation existed with the count of lactic acid bacteria. The mutual correlations between the figures thus obtained have been calculated. The figures for summer and for winter were compared. Upper limits for good butter have been established.

J. VAN BEYNUM en J. W. PETTE, Onderzoekingen over zuring en aromavorming bij practijkzuursels, in gebruik bij de boterbereiding, en methoden van onderzoek van zuursels. (Investigations on souring and aroma formation in butter starters). Verslagen van landbouwkundige onderzoekingen **47,** 1, 1941 en Jaarverslag Proefzuivelboerderij over **1940,** p. 59.

The rate of acid formation is estimated in sterilised milk which has been inoculated with 0.0067 % of the starter and incubated at 21° C. in a water-bath. The interval needed for the acidity to increase from 40 tot 60 ml 0.1 n per 100 ml is measured. In this interval the curve representing the increase of acidity is nearly a straight line and extra- and intrapolation of the titration data is permitted. With good starters the increase in acidity will take 2.0 to 2.3 hours.

The best aroma production occurs in starters with a not too rapid citric acid decomposition and a weak reduction of acetoin and diacetyl to butylene glycol. In this case the production of C_4-compounds is high. The reduction to glycol begins when the citric acid is still present and continues after all citric acid has been fermented. The authors introduce a new characteristic number R, the reduction percentage = the percentage of C_4-compounds present in the form of butylene glycol. R increases with time as HAMMER's creatin-test may indicate also.

In the starters tested there was a great variance in the rate of increase of R. At the onset of the total decomposition of citric acid R was 9—100 %, a week later it varied from 13—100 %. For a good starter R must be low. When the decomposition of the citric acid is too rapid, the quantity of C_4-compounds is low and reduction is strong. With starters composed of pure cultures of aroma betacocci and lactic acid streptococci, the reduction is always low. The formation of the C_4-compounds and most of the acetic acid comes to a close when the citric acid is wholly fermented.

K. HOLWERDA, Over den invloed van het diacetylgehalte van de boter op de beoordeeling van de reuk en smaak hiervan bij de boterkeuring van den Bond van Coöperatieve Zuivelfabrieken in Friesland. (On the influence of the diacetyl content of butter on the score). Officieel orgaan van den Algemeenen Nederlandschen Zuivelbond **36,** 608, 619, 1941.

On the average unsalted butter kept 7 days at 14° C. with a high diacetyl content scored highest. When the diacetyl content was low the butter scored low and more faults were observed. It seems that 0.8 mg diacetyl per kg is the under limit for good butter. After keeping the butter for a fortnight at 14° C. the tendency is still the same, but diacetyl and score correlate less closely, because in many samples faults begin to develop. In salted butter the diacetyl content is lower.

J. van Beynum en J. W. Pette, De waarde van de kreatine proef voor de praktijk. (The value of the creatin-test for practical use). Weekblad voor zuivelbereiding en handel **49**, 41, 1943.

The use of the creatin-test for the periodical control of starters in the dairy is advised. The test should be carried out on three consecutive days in the same sample, kept at 20 or 21° C. With a starter of good flavor the test must be negative after three days; otherwise the reduction of the aroma substance is too strong. The importance of using weakly reducing starters was proved. In fact butter of soured cream made with an aromatic starter had a very good flavor and contained 1.4—2.6 mg diacetyl per kg butter. With a strongly reducing starter, however, butter without flavor was obtained, which contained merely 0.3 mg diacetyl. However, when the butter is contaminated the effect of a good starter may be nullified as yeasts may reduce the aromatic compounds.

J. van Beynum en J. W. Pette, De verstrekking van zuursels voor de boterbereiding. (The supply of starters for the preparation of butter). Weekblad voor zuivelbereiding en handel **49**, 181, 1943.

Aromatic starters need a careful treatment. Contamination with bacteriophages may affect the acid formation and together with the presence of other lactic acid bacteria this may hamper the aroma formation.

S. G. Wiechers en H. Blumendal, Het verband tusschen de kwaliteit van de boter, hare bereidingswijze en hare bacteriologische kwaliteit. (The relation between the quality of butter, its mode of preparation and its bacteriological quality). Handelingen van het Genootschap tot bevordering van melkkunde **1940**, II, p. 12.

It has been studied in what measure the quality of butter correlates with the number of lactic acid bacteria in the butter, the starter, the pasteurised cream and the soured cream. No correlation could be found in so far as the butter was not heavily contaminated. The starters were tested and the rate of acid formation was determined by titration after 20 hours incubation at 15° C. A presence of noxious yeasts, molds and lactic acid bacteria may be detected microscopically in the ripe starters, kept at 35° C. for 2 days. The judging of the starters is of great importance for the control of the butter.

J. van Beynum en J. W. Pette, Het aantoonen van gasvormende bacteriën in kaas. (The detection of gas forming bacteria in cheese). Verslagen van landbouwkundige onderzoekingen **48**, 765, 1942 and Jaarverslag Proefzuivelboerderij over **1942**, p. 35.

Early gas formation in cheese is caused by coliform bacteria,

which ferment the lactose still present in this stage. Their number is estimated by means of Mc.CONKEY's technique. Subsequent gas formation may be caused by *Cl. tyrobutyricum*, *Propionibacterium Shermanii* or *Lactobacillus bifermentans*, all capable of fermenting lactates.

The number of spores of *Cl. tyrobutyricum* is estimated with a fermentation test by inoculating the cheese in dextrose-peptone-broth and cultivating under anaerobic conditions at 30° C. Use is made of glass tubes which are evacuated. When the tubes are opened the gas should be explosive and the liquid should smell of butyric acid. The propionic acid bacteria are to be estimated according to the above abstract and *Lactobacillus bifermentans* (see below) is detected by inoculation of the cheese in peptone-K_2HPO_4-2 % calcium lactate medium in tubes, which are evacuated afterwards. Incubation at 30° C. A slow fermentation occurs; the fermentation gas consists of nearly equal parts of H_2 and CO_2 and the culture medium obtains a sweet odour. The fresh medium has a pH of 5.6. It is more selective if the pH is 4.5, but the development of the bacteria is then very slow.

For inoculation of the tubes dilutions of the cheese are made (0.1 g—0.000.000.1 g) in sterile water after solving the cheese in a warm solution of 2 % sodium citrate. From each dilution 2 or 3 tubes of each medium are inoculated. The paper is illustrated with many photographs of cheeses.

J. VAN BEYNUM en J. W. PETTE, Propionzuurbacteriën in Goudsche en Edammer kaas. (Propionic acid bacteria in Gouda and Edam cheese). Verslagen van landbouwkundige onderzoekingen **47**, 1101, 1941 and Jaarverslag Proefzuivelboerderij over **1941**, p. 23.

Countings of these bacteria in cheese were made by inoculating various dilutions of the cheese in yeast autolysate-peptone-sodium lactate-agar or silica gel. The bacteria may be easily recognised by the size and shape of their colonies (seed- and lens-shape). Generally Edam cheese contains a merely small number of propionic acid bacteria, whereas in Gouda farm cheese large numbers could be found, although in a few cases in cheese of this type they also occurred in a low number. This great variety appeared to be due to the pH of the cheese.

In a series of experiments with cheeses of varying pH it appeared that the number of propionic acid bacteria was higher when the pH was higher. This correlation was rather close and the data about this correlation and about the natural rise of pH in the cheese during keeping offered the means to foretell the grade of development of the propionic acid bacteria. If the pH of a cheese of one day is under 5.0 no growth will take place, if over 5.1 then an abundant growth will ensue. If the pH of a cheese of 3 weeks is below 5.1 no growth will occur, if it is 5.2—5.3 their number will rise to some

hundreds of thousands; if it is above 5.3 millions will be found afterwards. No growth is observed until the 15th day and it may last till the 9th week at a temperature of 18° C.

The propionic acid bacteria seem to be of no importance for the cheese-ripening process. If present in a large number they will give rise to a subsequent blowing of the cheese. For the formation of a small number of gas holes in the cheese their number has to be merely moderate. As the fermentation gas (CO_2) is water soluble the holes can only be formed when small preformed holes occur in the curd, due to included air or to fermentation gas from *E. coli*.

J. W. Pette en J. van Beynum, Boekelscheurbacteriën. („Boekelscheur" bacteria). Verslagen landbouwkundige onderzoekingen **49**, 315, 1943 and Jaarverslag Proefzuivelboerderij over **1942**, p. 181.

The properties of a bacterium, often found in Netherland cheese causing gas formation have been studied. Generally its action in cheese is not very pronounced, but in some cases it causes serious subsequent blowing. This organism, *Lactobacillus bifermentans*, originally discovered and described but not named by Boekhout and Ott de Vries is a duplo-rod form. The colonies in yeast autolysate-sodium lactate agar are shaped like those of the propionic acid bacteria but are much smaller. The agar is torn by the gas development. It shows all properties of a lactic acid bacterium. In sugar media the pH decreases to 3.4—3.9. Inactive lactic acid if formed from hexoses, maltose, rhamnose, sorbitol and mannitol. Lactose, sucrose, raffinose, pentoses, glycerol, starch, dextrine, salicin and dulcitol are not fermented. Lactates are fermented and decomposed to acetic acid, alcohol, CC_2 and H_2; during this process the pH increases. Thus in a sugar medium two dissimilation processes may take place simultaneously. When, however, the formation of lactic acid is so rapid that the pH sinks below 4, the lactate is not decomposed. Only when the pH is kept at a higher level by addition of a buffer mixture, the decomposition of lactate will set in and then the bacterium performs two different dissimilation processes one after the other. Hence the name *Lactobacillus bifermentans* has been chosen.

J. van Beynum en E. A. Vos, Kan men „laat-los" met bromaten bestrijden? (Can subsequent blowing be prevented by bromates?). Weekblad voor zuivelbereiding en handel **49**, 229, 1944.

The possibility of preventing butyric acid fermentation in Gouda and Edam cheese by means of oxidising agents has been studied. Danish investigators had drawn the attention tot bromates and iodates. In accordance with the Danish results the authors found that bromates and iodates in very low concentrations inhibited

the growth of pure cultures of butyric acid bacteria in artificial media. Chlorates did not even in a concentration of 0.1 %. The three salts, however, were without any effect in cheese. Even 150 g bromate per 100 l of cheese milk did not prevent the butyric acid fermentation in the cheese experiments.

It appeared that this had to be ascribed to an early decomposition of the bromates and iodates in the cheese by lactic acid bacteria. Further it was shown that both salts have a retarding effect on the lactic acid fermentation, which is a disadvantage in cheese making. Antibut D (trade name of a substance to be used in preventing the butyric acid fermentation), however, was effective. Analyses showed that it contained 34 % $KBrO_3$ and 64 % KNO_3. It is the nitrate that prevents the butyric acid fermentation in cheese, not the bromate. Experiments were carried out in which even 2.5 g KNO_3 per 100 l was effective in preventing the butyric acid fermentation. This is in accordance with earlier experiments of Boekhout and Ott de Vries.

B. van Dam et J. G. Warffemius, Rapport entre la teneur en humidité du lait sec maigre (provenant de lait écrémé) et le développement d'une flore microbienne dans ce lait. (Relation between the moisture content of milk powder (derived from skimmed milk) and the development of micro-organism in this milk). Antonie van Leeuwenhoek **10, 123, 1944—1945.**

Experiments have been carried out with the aim of following the number of germs and the moisture content of milk powder when kept at various degrees of relative moisture. Initially the milk powder has been stored in layers of varying thickness. Later the samples of different fabrication have been stored in thin layers. When the milk powder is stored in surroundings the moisture of which it can absorb, than initially a strong decrease in the total number of germs will occur. Subsequently during a long period the number of germs remains fairly stable and increases anew, when the moisture content has attained a definite level. Below this critical moisture content the development of a considerable microbial flora is not possible. The critical moisture content does not depend on the vapour pressure of the air out of which the powder has absorbed water. The moisture content which allows the occurrence of visible growth of moulds is all the lower, the lower the relative moisture is whereat the powder is kept stored. At a relative moisture of 100 %, 90 % and 85 % moulding occurs before the moisture content of the milk powder is in equilibrium with the moisture of the surrounding air. At a relative moisture of 75 % the equilibrium has already set in before the development of moulds. Before attaining at the equilibrium at a relative moisture of 75 % some milk powders only increased in moisture after a much longer period and then up to a content of about 20 %, and

then that content decreased and attained a normal value, corresponding with that of other powders, which absorbed the water directly up to that normal content.

J. J. GHIJSEN, Beschrijving van proefnemingen op laboratorium, semi-technische en technische schaal met een versneld vlasroot-procédé. (Description of experiments on laboratory, semi-technical and technical scale with a procedure for accelerated flax retting). Mededeeling Nr. 64 van het Vezelinstituut T.N.O. Delft, 1942.

A flax-retting process has been worked out, by which a considerable shortening of the retting-duration can be obtained with respect to the ordinary hot water method. In this process the flax is inoculated with a certain volume of retting-water, obtained from a former retting, which has been aerated previously.

A. D. J. MEEUSE, Ervaringen met een versneld vlasrootprocédé in de praktijk. (Experiences with the method for accelerated flax-retting when applied practically). Mededeeling Nr. 71 van het Vezelinstituut T.N.O. Delft, 1942.

The advantages effected by the shortening of the retting-duration in using the quickened flax-retting process according to GHIJSEN consist chiefly in a more regular distribution of the work. The tending of the installation necessary for the GHIJSEN method is very simple. The quality of the ribbon obtained by the GHIJSEN method (starting from various kinds of flax) is practically equal to that of the classical flax-retting method.

A. J. KLUYVER, De microbiologische grondslagen der voedsel-conserveering. (The microbiological basis of the conservation of food). Chem. Weekblad **38**, 383, 1941; Pharmaceutisch Weekblad **77**, 1370, 1941.

The paper contains chiefly a discussion of the action of high and low temperatures and more especially the harmful action of high temperatures on bacteria. The loss of the reproductional power is taken as a criterium for the death of the bacteria. By means of graphs the letality of the bacteria and the spores as a function of time at various temperatures is visualised. Moreover of importance are: the original number of microbes, the state of maturity of the spores present, the chemical composition and especially the pH of the surrounding medium. Some results arrived at under practical conditions are discussed.

Besides this the advantages or disadvantages of temperatures beneath and just above the freezing point are discussed. Here as well the initial number of microbes is of importance and also the degree of moistness.

The influence of carbon dioxide, ozonising, drying and conservation by means of salt, sugar and acids is shortly discussed.

MYCOLOGY AND PLANT PATHOLOGY

H. A. DIDDENS und J. LODDER, Die Hefesammlung des „Centraalbureau voor Schimmelcultures", Beiträge zu einer Monographie der Hefearten, II Teil, Die anaskosporogen Hefen, zweite Hälfte. (The collection of yeasts of the „Centraalbureau voor Schimmelcultures", II part, The anascosporogenous yeasts, second half). N.V. Noord-Hollandsche Uitgevers Maatschappij, Amsterdam, 1942.

„Die anaskosporogenen Hefen, zweite Hälfte" gives the classification of the *Mycotoruloideae*, the second subfamily of the *Torulopsidaceae*. The *Mycotoruloideae* are characterized by the ability to form pseudomycelium (in some cases also true septate mycelium) on which the blastospores generally are produced in a typical manner. Chlamydospores and arthrospores may be present. In the first chapters the authors give a general review of the subdivision of the *Mycotoruloideae* accepted by other workers in this field, a discussion of the systematic position of the *Mycotoruloideae* within the *Fungi imperfecti*, the definition of the genera of the *Mycotoruloideae* accepted by themselves, and a discussion of the dissociation phenomena in the *Mycotoruloideae* in relation to their significance for taxonomy.

The investigation was extended over 384 strains, isolated from human and animal pathogenic material as well as from saprophytic material.

Based on the evidence obtained the authors divide the *Mycotoruloideae* in 3 genera: *Candida*, *Brettanomyces* and *Trichosporon*

The genus *Candida* is characterized by round, oval or elongated cells and by the lack of ability to produce arthrospores. 25 species and 8 varieties were accepted, of which 6 are new species (*C. catenulata*, *C. japonica*, *C. Melinii*, *C. robusta*, *C. Scottii*, *C. tenuis*) and 4 new varieties (*C. Guilliermondii* var. *nitrophila*, *C. heveanensis* var. *curvata*, *C. pellicosa* var. *cylindrica*, *C. tropicalis* var. *Rhagii*).

The genus *Brettanomyces* possesses ogivally pointed, besides round to oval or elongated cells. The pronounced acid production causes a rapid die-off of the cultures. This genus contains only 4 species and 2 varieties. The treatment of this genus is based on data obtained from the publication of CUSTERS.

The genus *Trichosporon* is characterized by the production of blastospores and arthrospores. True mycelium as well as pseudomycelium is present. It forms an intermediate genus between *Candida* and *Geotrichum*. The relation between the number of arthrospores and blastospores may, even in a same strain, be very variable. The authors divide the genus *Trichosporon* into 6 species and 1 variety. Descriptions are given of the new species *Trichosporon capitatum* and *Trichosporon fermentans*.

The work is especially of great importance in that clarity and unity has been established in the taxonomy of a group in which chaotic and scattered literature has brought confusion.

T. Hof, On the identity of *Torula cremoris* Hammer et Cordes with *Candida pseudotropicalis* (A. Cast.) Basgal. Antonie van Leeuwenhoek **9**, 77, 1943.

Torula cremoris Hammer et Cordes obtained from the „National Collection of Type Cultures" in London has been studied. Its characteristics were in good agreement with those described by Hammer and Cordes. A test for pseudomycelium was unmistakably positive. The strain is claimed to be identical with *Candida pseudotropicalis* (A. Cast.) Basgal.

F. H. van Beyma thoe Kingma, Ueber einige Formen von *Verticillium dahliae* Klebahn. (On some forms of *Verticillium dahliae* Klebahn). Antonie van Leeuwenhoek **6**, 33, 1939—1940.

Verticillium alboatrum and *Verticillium dahliae* can be sharply distinguished. *Verticillium album* forms only dark coloured mycelium which may swell into black mycelial knotted masses; moreover the older conidiophores are coloured brown at the base, whilst chlamydospores and pseudosclerotia are lacking. *Verticillium dahliae* develops many pseudosclerotia. Generally the mycelium mats will take on a black colour much more rapidly than in *V. alboatrum* which in pure culture often during a long period merely forms white mats. *V. dahliae* frequently occurs on many plant species.

New forms of *V. dahliae* are described: *V. dahliae* Klebahn forma *zonatum*, *V. dahliae* Klebahn forma *cerebriforme* and *V. dahliae* Klebahn forma *restrictum*. *Verticillium dahliae* is thus a group form such as it is known in *Penicillium*.

F. H. van Beyma thoe Kingma, Beschreibung einiger neuer Pilzarten aus dem Centraalbureau voor Schimmelcultures, Baarn (Nederland). V. Mitteilung. (Description of some new species of fungi from the Centraalbureau voor Schimmelcultures, Baarn, Netherlands. VI. Communication). Antonie van Leeuwenhoek **6**, 263, 1939—1940.

The following fungi were described anew: *Emericellopsis terricola*, *Emericellopsis terricola* var. *glabra*, *Penicillium euglaucum*, *Penicillium baarnense*, *Penicillium novae-zeelandiae*, *Bisporomyces chlamydosporis*, *Phialophora aurantiaca*, *Phialophora lutea-olivacea*, *Graphium Cartwrightii*, *Oospora colorans*, *Spirotrichum musae* and *Pestalotia natalensis*.

F. H. van Beyma thoe Kingma, Beschreibung einiger neuer Pilzarten aus dem Centraalbureau voor Schimmelcultures, Baarn (Nederland). VII. Mitteilung. (Description of some new species of fungi from the Centraalbureau voor Schimmelcultures, Baarn, Netherlands. VII. Communication). Antonie van Leeuwenhoek **8**, 105, 1942.

The following fungi are described anew: *Arachniotus dankaliense*, *Penicillium ingelheimense*, *Phoma suecica*, *Margarinomyces decumbens*, *Phialophora atra*, *Phialophora cyclaminis*, *Tritirachium cinnamomeum*, *Tritirachium roseum* and *Cephalosporium lanoso-niveum*.

F. H. van Beyma thoe Kingma, Beschreibung der im Centraalbureau voor Schimmelcultures vorhandenen Arten der Gattungen *Phialophora* Thaxter und *Margarinomyces* Laxa, nebst Schlüssel zu ihrer Bestimmung. (Description of the species of the genera *Phialophora* Thaxter and *Margarinomyces* Laxa present in the Centraalbureau voor Schimmelcultures next to a key for their identification). Antonie van Leeuwenhoek **9**, 51, 1943.

Phialophora and *Margarinomyces* are hard to distinguish. Therefore both genera have been treated monographically. 17 species of *Phialophora* and 6 species of *Margarinomyces* have been described. A key for their identification has been composed.

Y. van Koot, Viruszuivering en wat zij ons leert omtrent den aard van het virus. (Virus-purification and what it tells us about the nature of the virus). Tijdschrift voor Plantenziekten **46**, 97. 1940.

A comparative study was made of the purification of the ordinary tobacco mosaic virus and the single streak virus of the tomato. The following methods were used: the method of Stanley; filtration through celite and separation of the virus by different saturations with $(NH_4)_2SO_4$; that of Bawden and Pirie; heating to 70° C. and separation of the virus by adding HCl; and that of Rischkov and Gromyko; removal of the pigments by treatment with coal. As result a quick method was obtained by using plants grown under moist conditions in the shade, so that the juice did not contain too much pigment. This juice was filtered through celite, the virus precipitated with 40 % $(NH_4)_2SO_4$ followed by a single treatment with 1 % or 2 % coal, all at pH=7. Virus needles are only obtained when the virus is quite free of pigment and when $(NH_4)_2SO_4$ is added. The needles of the tomato single streak virus are somewhat shorter (15 μ) than the needles of the ordinary tobacco mosaic virus (20—25 μ). The virus-activity of the tomato virus, as measured by local lesions on *Nicotiana glutinosa* remained intact during a long chemical purifying process, while the activity of the tobacco virus decreased to a considerable extent.

J. G. Oortwijn Botjes, De toepassing van een beschuttende enting als middel ter bestrijding van virusziekten bij de aardappelplant. (The application of a protective inoculation as a means of control of virus diseases of the potato plant). Tijdschrift voor Plantenziekten **46,** 181, 1940.

The presence of mild mosaic in the variety Industrie protects this variety for infection with strong mosaic. Inoculation with both were made on the variety Eigenheimer with same result. The damage on Eigenheimer of light mosaic is, however, still such that it is not advisable to use its protective power for practical purposes. The danger of the use of protective virus inoculations is the occurrence of complex diseases. The X-virus of Irish Cobbler grafted on healthy Eigenheimer resulted in healthy plants, after grafting on mild or strong mosaic Eigenheimer a complex disease developed, the plants remained small, and the leaves were very crinkled. The reverse inoculation had no result. The X-virus present in Eersteling, Franschen, and Magdeburger Blaue inoculated by grafting on mild or strong mosaic Eigenheimer gave the same complex disease. As the X-virus is not transmitted by Aphidae, the complex disease does not occur in practice. A weak form of the X-virus also gave the same complex disease. A second complex disease is dwarf mosaic, which developed after grafting of strong mosaic on light mosaic Eigenheimer.

J. G. Oortwijn Botjes, De invloed van bladrolziekte op de opbrengst van verschillende aardappelrassen. (The influence of leafroll disease on the yield of different potato varieties). Tijdschrift voor Plantenziekte **47,** 25, 1941.

The yield of 16 potato plants of different varieties inoculated by grafting with leafroll was compared with that of 16 healthy ones. The loss in yield was very different with regard to the different varieties used, and greatest with the variety Paul Kruger (President) being 84,6 % and none with the variety Up to Date. The tubers are smaller and especially their specific gravity is less. Of a small number of varieties the yield of naturally infected potatoes was compared with that of healthy ones giving not quite the same results as that of the grafted ones.

C. Mastenbroek, Enkele veldwaarnemingen over virusziekten van lupine en een onderzoek over haar mozaikziekte. (Some observations in the field on virus diseases of lupin and an investigation on lupin mosaic disease). Tijdschrift voor Plantenziekten **48,** 97, 1942.

The symptoms of mosaic disease on *Lupinus luteus* occurring in the field are described, those of the secundary form of the disease on plants grown from virus containing seeds next to those of the primary form of the newly infected crop. The disease could

not be transmitted on the test sortiment of 8 varieties of american beans, but the inoculation on 2 dutch varieties of *Phaseolus vulgaris* was successful. Good results were also obtained on 3 varieties of *Pisum sativum*, on *Vicia faba*, on *Lathyrus odorata*, on *Trifolium pratense* and on *Lupinus albus*, but no results were observed on *Trifolium repens* and *Trifolium hybridum*, on *Lupinus mutabilus*, on *Soja hispida* and on *Nicotiana* spp. The coloured flowers of diseased *Lathyrus* were ,,broken". Seed transmission occurred on lupin for 5 %, but no seed transmission could be proved on a variety of *Phaseolus*. Between 60° C. and 70° C. the virus was killed. A dilution of 1/600 was still active. As the virus could not be transmitted to *Nicotiana* or to the test sortiment of *Phaseolus*, it is not identical with an already known virus. Therefore it should be called Lupinus virus 1. A mosaic virus occurring on *Lupinus albus* was not identical with it.

H. M. Quanjer, Bijdrage tot de kennis van de in Nederland voorkomende ziekten van tabak en van de tabaksteelt op kleigrond. (Contribution to the knowledge of diseases of tobacco occurring in the Netherlands and of the culture of tobacco on clay soil). Tijdschrift voor Plantenziekten **49**, 37, 1943.

Mosaic is generally present. It was proved again that the disease is not transmitted with seed. After handling the seedlings with hands, which had been in close contact with old dried, diseased tobacco, 17 % of the plants became diseased. Control measures are: destruction of all rests of old plants in the fields, disinfection of the hands during work. *Solanum nigrum* may be a source of infection. Next to mosaic a second virus disease occurred generally. It resembled ,,ringspot" but differed from it through the formation of typical stripes along the stem. The name ,,ring and stripe" disease is suggested. The symptoms are recorded in detail. The disease could be transmitted by sap inoculation. The virus is destructed at 80° C. during 10 minutes. The disease can be transmitted from the soil of seed-beds, seeds sown in diseased soil and handled very carefully gave 10 % of diseased plants. All varieties of tobacco used in the Netherlands as well as *N. macrophylla* and *N. rustica* were susceptible.

D. Noordam, Over het voorkomen van ,,spotted wilt" in Nederland. (The occurrence of ,,spotted wilt" in the Netherlands). Tijdschrift voor Plantenziekten **49**, 117, 1943.

Spotted wilt was found on *Richardia africana* Kunth and could be transmitted by sap inoculations to *Solanum lycopersicum*, *Solanum capsicastrum*, *Hippeastrum hybridum*, *Nicotiana glutinosa*, *Gloxinia hybridum*, *Pisum sativum*. The symptoms on all these plants are described.

J. Temme, Natrot bij tabak (*Nicotiana tabacum*). (Soft rot in tobacco, *Nicotiana tabacum*). Voorlopige mededeling over een nog niet eerder beschreven bacterieziekte van tabak. (Preliminary communication about an as yet undescribed bacterial disease of tobacco). T. voor Plantenziekten **49, 113, 1943.**

The symptoms are described of a disease in the leaves of tobacco observed for the first time in the Netherlands. Its investigation led to the detection of the causal bacterium up till then unknown. The organism appears to be related to *Erwinia aroideae* which causes a soft rot in bulb, stem and petioles of *Zantedeschia aethiopica* a.o. Sufficient terms are claimed to be present to bring this bacterium in a separate species. The name *Erwinia nicotianeae* is suggested. The disease caused by this bacterium is termed „soft rot of tobacco".

A. Jaarsveld, De invloed van verschillende bodemschimmels op de virulentie van *Rhizoctonia solani* Kühn. (The influence of various soil fungi on the virulence of *Rhizoctonia solani* Kühn). Thesis, Amsterdam 1940.

The investigations have been carried out chiefly by means of cotton-plugged culture tubes, thus under completely sterile conditions. Some experiments have been carried out in flower-pots. The influence on the parasitism of *Rhizoctonia solani* on seedlings of Chinese cabbage has been studied for a number of soil fungi, *viz.*, *Absidia spinosa*, *Cladosporium herbarum*, *Cylindrocarpon didymum*, *Penicillium expansum*, *Pyronema confluens* and two strains of *Trichoderma lignorum*. All these fungi appeared to act more or less antagonistically on the virulence of *Rhizoctonia solani*, as well in respect of the germination as of the seedlings. In the presence of 2 to 4 antagonistic soil fungi the total antagonistic action was stronger than the action of a single one. The addition of *Cylindrocarpon* to two or three of the others made the antagonism decrease somewhat, although *Cylindrocarpon* by itself acted antagonistically. In the pot experiments rice cultures of the fungi have initially been used. It appeared, however, that rice cultures of saprophytes as well acted injuriously, probably on account of the toxic substances which had developed in them. When agar cultures had been used as inoculum the results nearly completely corresponded with the experiments under sterile conditions.

The influence of temperature on the linear growth and dry weight of the various fungi was studied; minimum, optimum and maximum temperatures were ascertained. The antagonistic influence of *Absidia*, *Cylindrocarpon*, *Pyronema* and *Trichoderma* appeared strongest at the optimum temperatures of these fungi. Inversely at temperatures which favoured the development, the antagonism was not always very strong. For *Trichoderma* strain B the area of optimal temperature for the growth of the fungi was wider than for the maximal antagonistic action.

The antagonistically acting agens appeared not to be confined to the living fungi. The filtrates as well acted antagonistically. When *Rhizoctonia* was inoculated in various dilutions of the filtrates, growth was most strongly inhibited in undiluted filtrates. As a rule no pseudosclerotia were formed in undiluted filtrates, few in those that were slightly diluted and many in the strongly diluted filtrates. The filtrates as well appeared to act antagonistically on the virulence of *Rhizoctonia* against the seedlings.

In combinations of *Rhizoctonia* with the other fungi in petri dishes and drop-cultures various forms of inhibition occurred. No epiparasitism, however, has been observed.

K. Hartsuijker, Het wetenschappelijk onderzoek van fungiciden. (The scientific research of fungicides). Thesis, Amsterdam 1940.

The fungi used in this investigation were: *Venturia inaequalis*, *Phytophthora infestans*, *Cladosporium fulvum*, *Septoria apii graveolentis*, *Ascochyta pisi*, *Helminthosporium sativum*, *Botrytis cinerea*. The protective fungicidal action has been determined by placing spore suspensions on the dried chemical preparations, the direct action by incorporating the spores in the different dilutions of the preparations and the lethal effect by bringing the spores into contact with the preparation for some time, removing them again by centrifugating and sowing them out on cherry extract.

The experimental work falls into three groups: 1) The toxicity of five metal salts, *viz.*, $CuSO_4$, $HgCl_2$, $CdCl_2$, $NiSO_4$ and $ZnSO_4$. In this case the direct-fungicidal and the lethal effect were determined. The salts examined were found to differ a great deal in these respects. It was also found that the order of the fungicidal action of these salts is not the same in the fungi investigated. $ZnSO_4$ proved to be least toxic and hardly lethal. The strongest effect was observed in the case of $CuSO_4$ and $HgCl_2$, there being very little difference between the two. Only $HgCl_2$ was considerably more lethal than $CuSO_4$. $CdCl_2$ was found to occupy an intermediate position, since its toxicity was generally less, but never more than that of $CuSO_4$. In the case of $NiSO_4$ the results obtained with the various fungi were widely divergent. To *Botrytis cinerea* it was the most toxic of all salts, while it was also toxic to the indirect germination of *Phytophthora infestans*. The results obtained with $NiSO_4$ might explain the widely divergent data supplied in the literature on the effect of nickel salts. During the determination of the lethal effect of $NiSO_4$, it was observed that higher concentrations were less lethal than lower concentrations. This phenomenon, which was also observed for $CuSO_4$ in the case of *Botrytis cinerea*, could not be explained.

2) The toxicity of a number of sulphur compounds, *viz.*, three different polysulphides (Ca-, NH_4- and Ba-polysulphide), a monosulphide (Ca-monosulphide) and a colloidal sulphur (colloidal

bentonite sulphur). A marked difference between the direct- and the protective-fungicidal action was only observed in the case of the three polysulphides. The strong direct-fungicidal action of the polysulphide is of great importance in practice (extermination of existing infections). The protective-fungicidal action of the polysulphides was also better than that of the two other types of sulphur preparations. At an equal percentage of total-substance the protective-fungicidal action of the colloidal sulphur was better than that of the monosulphide.

3) The toxicity of mixtures of polysulphides and lead arsenate. The materials used were polysulphides of Ca and NH_4 in combination with powdered lead arsenate and additional substances such as lime and ferrous sulphate. Lead arsenate was found to possess only a very slight fungicidal action. The fungicidal action of combinations of 1 % of polysulphide and respectively 0.1 %, 0.3 % and 0.5 % of lead arsenate was somewhat better than that of polysulphide alone, although the difference was only very slight. The quantity of lead arsenate added did not influence the fungicidal action to any appreciable extent. The destroying effect of the lead arsenate on the polysulphides is either very slight or entirely compensated for by the fungicidal action of the „soluble arsenic" formed. This fact is supported by practical data obtained from the literature.

D. Mulder, Biologisch onderzoek van grondontsmettingsmiddelen. (Biological investigation of soil disinfectants). Thesis, Amsterdam 1943.

The aims of this investigation were: *a*) to draw up a chemotherapeutical index for soil disinfectants. *b*) To develop experimental methods to obtain this index. *c*) To collect data concerning the decrease in effectiveness of a disinfectant brought about by contact with the soil. *d*) To determine a dosis curativa for disinfectants in soil. *e*) To test the usefulness of some new methods of soil disinfection.

The materials used were the ordinary seed- and soil-disinfectants. Besides these products special organic mercury-compounds could be desposed of for the study of the influence of certain admixtures, the study of the difference between ethyl- and phenylmercury compounds and the influence of different acid groups. The dosis toxica in relation to fungi and the dosis tolerata for seedlings were determined experimentally in order to calculate the chemotherapeutical index. The growth of *Pythium de Baryanum* in 0.5 % saccharose solution, without the addition of nutritional salts, satisfied as criterion for the toxicity. Proportionality appeared to exist between the rate of growth and the concentration of the poison. In the case of adsorption-experiments the growth of *Pythium* mycelium used for determination of toxicity, provided the possibility of an accuracy, which surpassed by far those of chemical

methods of defining the concentration. A low and consequently favorable index-figure obtained in the laboratory, however, often coincides with insufficient disinfectant qualities in soil.

Therefore other qualities than those, which can be studied in vitro in contact with fungi and plants, also influence the value of a mercury-compound as soil-disinfectant. One of these qualities is the adsorption of the compound in soil by which its working capacity decreases. The determination of its adsorption was carried out both in garden-soil and with adsorbent coal. From the dosis toxica before and after the adsorption an adsorption-factor can be calculated. The historical development of the seed- and soil-disinfectants shows a parallel with a decrease of adsorption. The phenylmercury-compounds are adsorbed ten times as strongly as the ethylmercury-compounds. The results with the various compounds is closely related with the intensity of attack in the experiment in question. Therefore only restricted mutual comparison is permitted. The application of formaldehyde as a dry powder, made by mixing it with infusorial earth or saw-dust, was a complete success: the disinfection was nearly as good as with formalin 4 %. Complete disinfection was obtained only with formaldehyde. On the other hand the highest increase in number of seedlings is reached with one of the mercury compounds.

SOIL BACTERIOLOGY

G. W. Harmsen, The influence of the method of sampling on the accuracy of the determination of bacterial numbers in the soil. Antonie van Leeuwenhoek **6**, 178, 1939—1940.

Tests were made to determine the influence of the sampling and the preparatory treatment of the samples on the counting of bacteria in soil. These proved that the present methods are very inaccurate and give rise to considerable variance in parallel determinations, to the extent even of rendering the effect of a perfected counting procedure quite negligible. For a better preparatory treatment of the samples a homogenisation method by means of a porcelain ball mill was worked out, by which means a suspension of the sample is made in water, which method has given satisfactory results.

F. C. Gerritsen, Enkele waarnemingen betreffende den invloed van de temperatuur op de nitrificatie en vastlegging van de stikstof. (Some observations concerning the influence of temperature on the nitrification and assimilation of nitrogen). Landbouwkundig Tijdschrift **54**, 573, 1942.

Laboratory tests were carried out, one series with a variety of constant temperatures, the other with varying temperatures in the open. 50 mg $(NH_4)_2SO_4$ had been added per 100 g of dry soil.

The balance and transformation of nitrogen were checked by periodical estimations of NO_3-, NH_4- and total nitrogen. Nitrification occurred even between 0° and 5° C. and at 5° C. a normal supply of nitrogen may be entirely nitrified within a fortnight. The experiment in the open showed that the nitrification process was fairly strongly influenced by temperature. When this process had arrived at its close the whole balance of nitrogen was dominated by strong periodical fluctuations in the assimilation of N by microbes. A reason for these fluctuations could not be presented.

Y. VAN KOOT, Grondontsmetting door stoomen en de beïnvloeding van het bacterie leven en de samenstelling van de grond. (Disinfection of soil by steaming and its influence on the bacterial life and composition of the soil). Landbouwkundig Tijdschrift **54,** 532, 1942.

Experiments were carried out in a cold winter under various moisture conditions. Directly after steaming the bacterial number was very low. After some weeks bacterial numbers had increased strongly and surpassed the original amounts by far. The numbers of nitrifyers increased much later when the temperature of the soil had risen considerably. The numbers of the protein decomposing bacteria did not increase so distinctly. The amount of water soluble nitrogen generally increased directly after steaming and this lasted up till the moment of increase of bacterial numbers. Then a distinct decrease in bacterial numbers occurred. When the soil had been steamed under too moist conditions or had been leached out after steaming, no increase of nitrogen occurred and the development of the bacteria was much slighter.

K. T. WIERINGA, Determination of the fertility of the soil by microbiological methods. Antonie van Leeuwenhoek **6,** 56, 1939—1940.

A review is given of the determination of soil fertility by microbiological methods. The quantitative and the qualitative method for the determination of the microflora were discussed as well as those methods in which micro-organisms are used for ascertaining the particular condition of a limiting factor in the soil. More especially the use of *Azotobacter* cultures for this purpose was mentioned.

E. G. MULDER, On the use of micro-organisms in measuring a deficiency of copper, magnesium and molybdenum in soils. Antonie van Leeuwenhoek **6,** 99, 1939—1940.

A description is given of some microbiological tests for the determination of plant-available copper, magnesium and molybdenum in soils. In these investigations *Aspergillus niger* and in a few cases

Azotobacter chroococcum and *Bacterium prodigiosum* were used. From the figures obtained it is revealed that soils on which the plants are suffering from the so-called ,,reclamation disease", have a much lower available copper content than those producing healthy crops. Soils on which the plants show the so-called ,,Hooghalen disease", have a very low content of available magnesium. From these results and from experiments with cereals it is concluded that the ,,reclamation disease" is caused by a deficiency of plant-available copper and that a deficiency of available magnesium is the chief cause of the ,,Hooghalen disease".

F. C. Gerritsen en Nora Blumendal, Een onderzoek naar de bruikbaarheid van de *Aspergillus*-methode voor de bepaling van phosphorzuur en kali in den grond. (An investigation of the suitability of the *Aspergillus* method for the estimation of phosphoric acid and potassium in soil). Verslagen van landbouwkundige onderzoekingen. **46, 219, 1940.**

F. C. Gerritsen und Nora Blumendal, Phosphat-Bestimmungen mittels *Aspergillus niger*. (Estimation of phosphate by means of *Aspergillus niger*). Antonie van Leeuwenhoek **6, 71, 1939—1940.**

The estimation of phosphate in soils according to the *Aspergillus* method of Niklas entails some difficulties. The production of acid by the fungus varying along with the content of phosphate of the medium, the pH of the medium varies too. The varying lime content of the soils influences the development of the fungus in two ways: *a*. by altering the buffering of the medium and *b*. by the action of calcium as a nutritive for the fungus. Certain humus substances may influence the amount of mycelium formed. By substituting 0.6 % ammoniumsulfate by 0.4 % urea the changing of pH in the medium is brought down considerably. By adding Ca nitrate a better buffering and independence of the Ca content of the soil is arrived at. The supply of a minute dose of humic acid (Na humate) removed a source of irregularity and caused in some cases an increase in mycelium of 9 to 40 %. By these means the maximum change in pH could be brought down from 1.8 to 0.37. In tests of soils of various origin the change in pH was on the average 0.14.

In the presence of potassium sodium salts (5 m. mol pro litre) appear to favour the development of mycelium (an increase up to 30 %).

It is claimed that the *Aspergillus* method belongs to the cheapest and most reliable methods for the estimation of the need of P and K in soils.

Jan Smit and E. G. Mulder, Magnesium deficiency as the cause of injury in cereals. Meded. van de Landbouwhoogeschool **46**, Verhandeling 3, 1942.

In order to ascertain the cause of the disease of oats and other

cereals occurring frequently on acid soils *Aspergillus niger* was used as a test organism for the estimation of plant available magnesium in soil. Its development in a magnesium free solution, where the soil investigated was the only source of magnesium, was recorded. A series of flasks with known amounts of the element served as a standard of comparison. It proved a satisfactory method to estimate the part of the total magnesium which may be assimilated by *Aspergillus niger*. Healthy soils appeared to contain 100 γ or more per 3 g of soil. A striking parallelism was found to exist between the occurrence of the disease and the amount of Mg available to the mould.

Marie P. Löhnis, The action of manganese on the development of *Aspergillus niger*. Antonie van Leeuwenhoek **10**, 101, 1944—1945.

The response of *Aspergillus niger* to the presence of manganese in the nutrient solution has been ascertained. In an initially acidified medium which kept up its acidity or in an unbuffered neutral medium which grew strongly acid, manganese appeared essential for sporangial development. In suitably acidified solutions KNO_3 as source of nitrogen induced slight sporangial development in the presence of 0.05 γ Mn, $NaNO_3$ of 0.025 γ Mn and NH_4NO_3 of 0.05 γ Mn in 40 ml of medium. In the acidified NH_4NO_3 medium the largest number of visually to be distinguished amount of sporangia could be ascertained. In acidified solutions with KNO_3 the yield of mycelium was higher in the absence of manganese than in cultures which in its presence had developed sporangia. Presumably the development of sporangia inhibits further development of mycelium. The weight in yield of mycelium in an acidified $NaNO_3$ solution in the absence of manganese was very significantly lower than in the presence of 0.005 γ Mn. In non-acidified nitrate solutions sporangia developed in the absence of manganese. No definite trend in the yield of mycelium could be ascertained. In unbuffered solutions with ammonium salts no sporangia developed in the absence of manganese. The response on various amounts of manganese was erratic. No definite trend in mycelial development could be ascertained. Solutions with an ammonium salt suitably buffered by $CaCO_3$ produced sporangia in the absence of manganese. They gave the highest yields in weight. The intake of manganese by the fungus on various media offers slight differences. The intake is slightly higher in weakly acid than in strongly acid solutions. The fact that manganese is only essential in a highly acid medium may not be ascribed to an action of the medium as such on the availibility of manganese and must be caused by differences in the metabolism of *Aspergillus niger*.

As *Aspergillus niger* is sensitive for traces of manganese only under highly acid conditions an *Aspergillus* standard will not be suitable for soil tests.

K. T. Wieringa, Landbouwkundige en Landbouwmicrobiologische problemen betreffende de sporenelementen (Oligopleronten). (Agricultural and soil microbiological problems concerning the micro-elements (oligopleronts)). Landbouwkundig Tijdschrift **56**, 303, 1944.

Problems concerning micro-elements (oligopleronts) in agriculture and soil microbiology are discussed. The name oligopleronic elements or oligopleronts is forwarded as apt for international use.

The paper is a review of the literature on some deficiencies in plant growth, their occurrence and causes in connection to soil conditions such as pH, Eh and structure. The grey speck disease (Mn-deficiency), magnesium deficiency, and reclamation disease (Cu-deficiency) are discussed.

The *Aspergillus* method as a means of testing the soil for these deficiencies is paid attention to.

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THE HYDRATION OF THE IX AND Vi ANTIGEN OF *BACTERIUM TYPHOSUM*

by

R. TH. SCHOLTENS

(Received September 28, 1945).

ARKWRIGHT (1, 2, 3) described the occurrence of the smooth form — rough form variation of bacteria. Associated with the change in colony form he reports the appearance of autoagglutinability and decrease of virulence. BRUCE WHITE (12, 13) ascertained that both modifications possess the same thermolabile antigens localised in the flagella, whilst they differ, however, in their body antigens. The smooth forms, which are not flocculated in 0.9 % NaCl, contain body antigens which induce granular agglutination and a sandy sediment with corresponding agglutinins. The surface of the less stable rough form is covered with other antigens inducing agglutination of much finer grade next to a muddy sediment. WEIL and FELIX (11) did not keep in view the latter form of agglutination, when they introduced the term O agglutination. BRUCE WHITE (12, 13) reserves the term O antigen for body antigens occurring in the smooth form and not in the rough; designating the antigen of the rough form he uses the sign ø. He carried out biochemical tests with bacterial suspensions of smooth and rough forms of various Salmonellae (14). The smooth form gave a stronger reaction with Molisch test (carbohydrate) than rough forms. So he drew the conclusion that bacteria in the rough form are externally of protein substance. The smooth form, however, is externally of carbohydrate substance. By heating at 100° C. in 0.2 n acetic acid he extracted substances from the smooth form that did not produce antibodies in rabbits; they gave good precipitation reactions with sera made from various Salmonella types, corresponding with the O agglutinations from the Salmonella types used in these tests. Protein tests (biuret and Millons) were negative, Molisch test strongly positive. These substances could not be extracted from corresponding rough forms, so they appeared specific for O antigen. Smooth strains of bacteria, which had been treated similarly, flocculated in 0.9 % NaCl. They allowed agglutination, although up to merely $^1/_4$ of the titre with rough

agglutinins, no longer with 0 agglutinins. In view of this he ascribes the difference between the smooth and rough forms to the presence of these extractable substances containing carbohydrates. These substances form the 0 antigen complex and by their hydrophilic character cause the stability of the smooth form in salt solution. The antigens of the rough form are also found in the smooth form, although covered by the 0 antigen, perhaps mechanically.

According tot KRUYT (6, 7, 8) a hydrophilic colloid derives its stability from two factors, *viz.*, hydration and charge. In order to obtain flocculation both must be, partly, eliminated. The charge is removed by means of an electrolyte, the hydration by increasing concentrations of alcohol. Increase of the alcohol concentration means the need of addition of less electrolyte for the induction of flocculation. The combinations of alcohol and electrolyte concentrations just allowing of flocculation are different for various types of colloids.

As a matter of fact the 0 antigens which have been discussed, are hydrophilic colloids (BRUCE WHITE (14)), so the bacteria will flocculate by various combinations of alcohol and electrolyte.

The stabilising hydrophilic antigen complex is serologically of different composition in various antigenic types of typhoid bacteria (SCHOLTENS (9)). It may consist out of the IX (XII) complex, or the Vi antigen (described by FELIX (5)) or both antigens together. In the following experiments the stability of the three antigenic varieties is tested in various concentrations of NaCl and alcohol and compared with the stability of the rough form. The influence of hydration of Vi, respectively of the IX antigen may be read from the degree of stability of these suspensions.

Technique.

The glassware was freed from grease and electrolytes by boiling with soda and further treatment with acid and steam. The alcohol was distilled over calcium oxyde. Undistilled the alcohol was found to contain small amounts of acids which disturbed the test (Prof. Dr REITH). The strength of the alcohol thus obtained was 95 volume percent. Mixtures of 90, 80, 70, 60, 55, 45, 30, 20 and 10 volume % alcohol were prepared according to the alcohol dilution tables of the Chemical Yearbook No. 3.

A 4 n solution of NaCl was prepared by solving 58.5 g NaCl in 250 ml distilled water. By adding water solutions with concentrations of 10, 25, 50, 75, 150, 200, 300, 500, 1000 and 2000 millimol NaCl per litre were prepared.

The bacterial suspensions were obtained by growing the strain to be tested on Roux bottles for 24 hours and shaking off with distilled water. The completely homogenous suspension was centrifuged for a few minutes at 1000 r.p.m. Thus the larger particles, among which bits of agar, were deposited. The suspensions freed from these particles, were poured in centrifuging tubes freed

from grease and electrolytes. Then they were washed twice and resuspended in boiled distilled water.

The strains examined.

Most of the strains of *Bacterium typhosum* were isolated at the „Rijksinstituut voor de Volksgezondheid". Antigenic variants of these originated either out of broth cultures unto which specific serum had been added, or were obtained by means of a Vi bacteriophage. Besides these strains I worked with the strain 0 901 of Dr FELIX and the strain Rauss of Dr RAUSS (the latter obtained from Dr HAKMAN of the laboratory of Prof. RUYS), both without H antigen. The H and Vi antigen were checked by agglutination with the corresponding sera. 0 antigen was determined by agglutination with Gärtner serum, or, when Vi antigen was also present, by means of an adsorption test. The strains were moreover tested with Millons reagent as applied by BRUCE WHITE (14). The rough strains gave a positive reaction. The strain containing the Vi antigen (no IX (XII) complex) also proved positive. The Vi antigen itself, however, is not positive for the Millons test. It may be assumed, therefore, that the surface of this form is not completely covered by the Vi antigen, part of it being covered by protein. So it may be expected that in alcohol salt mixtures the stability of this form would be affected. The form containing the IX (XII) complex (no Vi antigen) generally shows a negative reaction with Millons reagent. In this form the albuminous rough antigens are more completely, if not entirely covered by the 0 antigen.

One of the examined IX (XII) strains, strain 0 901 sec x possessed the IX (XII) antigen, but as it reacted positively with Millons reagent, it was apparently merely partly covered by this antigen. The remaining part, covered by the albuminous ø antigens, influenced the stability. This was proved by an acid agglutination test, its result being negative with the ordinary 0 901 strain and with strains of equal arrangement of antigens, but positive with this strain 0 901 sec x. Moreover this form was agglutinated by ø agglutinins.

This and similar strains were cultivated from commonly occurring (IX (XII)) forms by means of a specific bacteriophage X. The secondary growth arising after lysis by this bacteriophage is plated. Subcultures of a few of these colonies show the recorded characteristics (SCHOLTENS (10)). Apparently the grouping of the XI (XII) antigen on the surface of these bacteria is similar to the grouping of the Vi antigen on the surface of the Vi (no IX (XII)) form.

The flocculation of the strains examined.

It appeared necessary to eliminate the influence of the H antigen because alcohol and salt caused a flocculation in the presence of this antigen. It would have been preferable therefore to carry out all experiments with immotile strains (containing no H antigen).

However it is difficult to find a larger number of such strains. So I began by examining five suspensions of each antigenic type, wherein the H antigen was destroyed by heating (15 minutes at 100° C.). Some immotile strains were included, also 0 901. In a second experiment a few curves were made with unheated suspensions obtained from immotile strains.

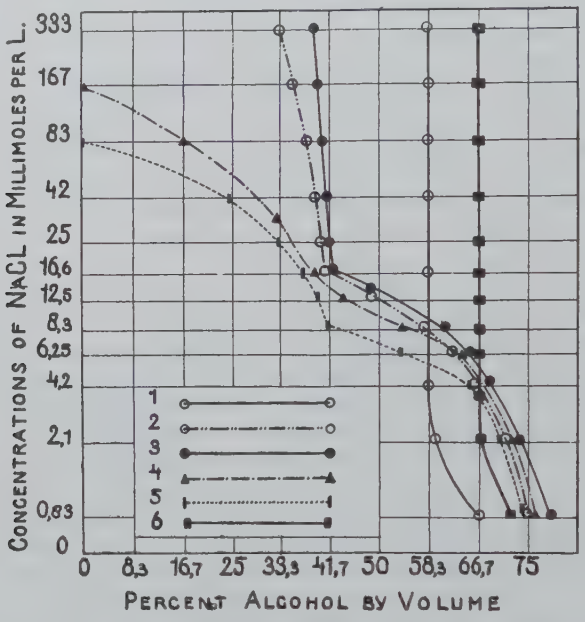

Fig. 1. Curves obtained with heated suspensions.

To obtain flocculation the following amounts of material were mixed: 0.25 ml salt solution, 0.25 ml bacterial suspension and 2.5 ml diluted alcohol. Readings were taken after 24 hours at room temperature. Fig. 1 and 2 present the curves obtained by plotting salt and alcohol concentrations, the dots marking the beginning of flocculation. On the abscis the alcohol concentrations of the mixtures are indicated, the distances are proportional to the logarithms of the concentrations. On the ordinate the NaCl concentrations are marked in millimoles per litre, the distances are proportional to the logarithms of concentrations. The dots marking the curves correspond to strains of various antigenic types. Table I explains their meaning.

In fig. 1 examples of curves are taken up which are obtained with heated suspensions. When suspended in pure water the rough form is flocculated by a NaCl concentration of about 100 millimol per litre, in a concentration of alcohol of 75 % by 1 millimol per litre. The curves 4 and 5 connecting these two points are not

uniform. This might be explained by a difference in the substances covering the bacterial surface. The type containing the IX (XII) antigen (no Vi antigen) — curve 1 — is flocculated in the same measure by alcohol in all higher salt concentrations. When, however, the salt concentrations are lower than 2 millimol, larger amounts of alcohol are needed. This may be explained by the fact that the

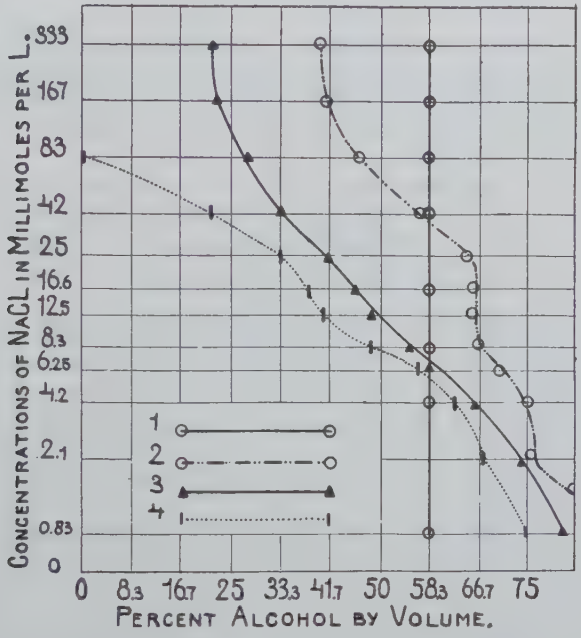

Fig. 2. Curves obtained with unheated suspensions.

IX antigen is strongly hydrated and possesses weak electric properties (DEKKER, VAN DER MEER and SCHOLTENS (4)).

Table I.
Key of figure 1 and 2.

Figure	Number of curve	Substances detected at the surface of the bacterial body	
		serologically	by Millons reagent
I	1	IX (XII)	—
	2,3	Vi	ø
	4,5	—	ø
	6	IX (XII)	—
II	1	IX (XII)	—
	2	Vi	ø
	3	IX (XII)	ø
	4	—	ø

The curves for bacteria possessing IX and Vi antigen agreed with the curve of the form containing only the IX (XII) antigens and no Vi antigen.

Strain 0 901 diverged somewhat in behaviour. Its curve runs parallel with those for other strains of similar antigenic structure (including some forms also H inagglutinable), but is shifted towards a stronger concentration of alcohol (curve 6).

The curves 2 and 3 for strains containing the Vi antigen as only stabilising antigen run parallel in the higher alcohol concentrations with the curves for the rough forms. At a concentration of about 40 % alcohol there is a steep incline. The antigenic variant is stable in lower alcohol concentrations. Hydration of the Vi antigen will furnish the explanation.

Only a few unheated suspensions have been examined. The results are taken up in Fig. 2. These suspensions yielded similar results, although in a few points they diverged in behaviour. The hydrophilic character of the Vi antigen appears most clearly in curve 2 produced by form Vi (no IX (XII)) which on the whole does not run parallel with the curve for the rough form — curve 4 —, but is shifted towards the higher concentrations of alcohol.

It may be noted that this form is flocculated in lower alcohol concentrations than the IX (XII) (no Vi) strains — curve 1 —. It might appear that Vi antigen differs in this colloid chemical respect from the 0 antigen. This, however, needs not necessarily be the case. It might as well be attributed to a more or less incomplete covering of the bacterial surface by the Vi antigen in the Vi (no IX (XII)) forms like it has been discussed above.

The form of 0 901 which is only partly covered by the IX (XII) antigen complex give rise to a curve — curve 3 —, which as well is shifted in the direction of the lower alcohol concentrations.

The curves described justify the assumption that the Vi antigen as well as the IX (XII) antigen are decidedly of the character of hydrophilic colloids.

Summary.

The Vi antigen as well as the IX antigen are hydrophilic colloids.

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INVESTIGATIONS CONCERNING THE SYMBIOSIS OF BACTERIA IN *TRIATOMA INFESTANS* (KLUG)

by

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Although a symbiosis with bacteria and fungi could be demonstrated in many insects with one-sided feeding, up till a short time ago this had not met with success in the family of the *Reduviidae* (BUCHNER (2)). In 1926, however, a paper was published by DUNCAN (5) in which he described the occurrence of bacteria in the alimentary tract of *Rhodnius prolixus*. This blood sucking insect was studied more closely by WIGGLESWORTH in 1936 (10). In the wall of the proventriculus he noted intracellular „feebly Gram positive", diphtheroid bacteria. Periodically (after blood sucking) part of these bacteria is set free in the intestinal lumen, and reach together with the blood the stomach and finally under some changes in form the end of the mid-gut. Further he gathered indications that the bacteria would produce a vitamine by means of which they might play a rôle in the metabolism of the bugs. Moreover he succeeded in obtaining on glucose agar a pure culture of the bacteria as far as they occurred freely in the intestinal lumen. He did not succeed, however, in growing the intracellular form of the bacteria, such as they occur in the young larva which had not yet sucked blood and in which the extracellular bacteria are not yet present. Neither could bacteria be grown out of the eggs or the ovaries. In the rectum of this insect no proventricular bacteria could be ascertained. DUNCAN found the rectum of the same insect sterile, just as was the case in a great many other insects which feed in all stages on blood.

The assumption of WIGGLESWORTH that the symbiotic bacteria had actually been obtained in pure culture was based on morphological similarity; stress was lain upon an equal series of transformations such as they occur in the bug and on the culture medium. According to the same author a similar symbiosis could be noted in *Triatoma rubrofasciatus* de Geer, *Triatoma infestans* Klug and *Eutriatoma Neiva*.

Liem (8) as well noted the diphtheroid, symbiotic bacteria in the proventriculus of *Triatoma infestans*.

Our investigation was taken up with the aim of studying in how far the features observed by Wigglesworth in *Rhodnius prolixus* would agree with those in the likewise blood sucking bug *Triatoma infestans*.

1. Methodology of the bacterial investigation.

The bugs under examination are killed in vapour of ether, stripped of their wings, sterilized externally (Zephyrol Bayer 10 %) and fixed in a Petri dish in paraffine (melting point about 55°C.). After the surface of the paraffine has been sterilized by flame the bugs are fixed in a way that merely the back remains raised above the coagulating paraffine.

Subsequently the bug is opened by means of boiled-out instruments, when needed under a binocular microscope, in a sterile physiological salt solution (0.9 % NaCl). Finally the organs which have to be examined more closely are prepared. Such organs are then either crushed on a slide for direct microscopical examination or a smear is made on an agar plate with 1 % glucose [1]) in order to obtain a culture of the bacteria if present.

These manipulations took from a quarter of an hour to an hour, according to the rapidity with which the organ or organs to be examined could be handled. Thus air born infection is by no means excluded. In order to reduce this risk some precautions were taken, *e.g.*, if the manipulations lasted for long, the instruments have been boiled out once again and the salt solution was replaced by a fresh one. At all events the result proved that considerable air infection only rarely occurred (in such cases the data have not been inserted in the table); apparently the manipulations have most often been carried out sterilly.

Another risk lay in the fact, that during the preparation of a definite organ the bacteria it might contain would infect other organs. By a suitable choice of the order in which the organs were removed this danger has been reduced as much as possible. Moreover in such cases, before proceeding to the preparation of another organ, some fluid out of the neighbourhood of the already removed organ was drawn up with a Pasteur pipette and streaked on glucose agar in order to ascertain afterwards an eventual infection.

By means of the data, partly summarized in the table, the following results were arrived at. Out of the proventriculus of the adult bug always morphologically similar bacteria (indicated in the following as proventriculus bacteria) could be grown in pure culture, *viz.*, small, somewhat bipolar, Gram negative bacteria.

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[1]) This nutrient medium was used by Wigglesworth. It turned out, however, that an addition of glucose is not necessary for the development of the proventriculus bacteria of *Triatoma infestans*.

Organ	Microscopically		Culturally		Other bacteria
	Proventriculus bacteria		Proventriculus bacteria		
	present	absent	present	absent	present
proventriculus					
a. of adult	3	—	7	—	3 [1])
b. of larva-blood	1	—	—	1	—
stomach					
a. of adult	1 (?)	—	3	—	1 [2])
b. of larva-blood	—	—	—	3	—
mid-gut [4])	1	—	—	—	1
rectum [5])	—	—	2 (?)	14	14 [3])
liquid of the penis [6])	—	—	1 (?)	3	2 [2])
ovary	—	—	—	3	1 [2])
vagina	1 (?)	—	2 (?)	1	—

[1]) *Viz.*, in three out of the 7 cases in which proventriculus bacteria had been traced by cultural means.

[2]) Bacteria differing morphologically from the proventriculus bacteria, *viz.*, duplococci and staphylococci.

[3]) Nearly always in samples of faeces collected in various ways large Gram positive cocci occurred which developed on the agar plates into minute colonies.

[4]) The part of the alimentary tract between stomach and outlet of the tubes of Malpighi.

[5]) Contents have been collected: *a.* by making the bugs defaecate. *b.* by compressing the abdomen. *c.* by means of a Pasteur pipette brought into the anus. *d.* by preparing the rectum out of the abdomen. The defaecation occurred (usually within 15 minutes) whilst the bug immediately after the feeding was held in a clip above a glucose agar plate without touching it. In order to prevent air infection the whole was kept covered by means of the upper lid of a sterile Petri dish.

[6]) The pale yellow evil-smelling liquid that could be collected by pressure out of the penis.

On glucose agar they slowly develop into greyish-white, glistening colonies with a trend to a notching of the margins.

These bacteria occur as well in the stomach and possibly in the part of the alimentary tract between stomach and outlet of the tubes of Malpighi, and in the rectum. Numerous colonies of another type always developed out of the contents of the rectum. A contamination by means of bacteria on the external surface of the abdomen cannot have caused this phenomenon, as an external sterilization proved without influence. In the testes and in the eggs bacteria were traced which were morphologically similar to the proventriculus bacteria. We did not succeed in cultivating these bacteria [1]). May be the proventriculus bacteria occur also

[1]) We tried to cultivate the bacteria out of the eggs on the following

in the penis of the bug. In the vagina a bacterium has been traced which is morphologically very much like the proventriculus bacterium, but differs considerably in form of the colony and in other respects (see furtheron). In the haemocoele no bacteria could be ascertained.

2. The proventriculus bacteria.

The air, dust or instruments of the laboratory room, when examined, proved free from these bacteria, thus any regularly occurring contamination from these sources is out of the question.

Are the morphologically similar bacteria which have been isolated out of the proventriculus of various bugs indeed identical? If this would be the case, it would be practically certain that the bacteria which occur in the proventriculus of the living bug had actually been isolated.

Wigglesworth assumes this to be the case, as he could ascertain in microtome sections of proventriculus and stomach from a few specimens of *Rhodnius prolixus*, fixed at various intervals after their feeding, a series of alternations in shape of the bacteria which he could note also in the isolated strains. We, however, could not note such a regularity in alternations of shape in the proventriculus bacteria from *Triatoma infestans* isolated by us.

We arrived, however, at the certainty that we handled the actual bacteria by studying the biochemical and agglutinogenic properties of 4 strains of proventriculus bacteria isolated out of 4 different bugs.

a. Biochemical properties.

The strains [1]) behaved as follows (compare also Beeuwkes and Bannink (1)) when tested with the following chemicals: saccharose, maltose and mannite with glutaminic acid, no fermentation; lactose and glucose with meat broth or with glutaminic acid, no fermentation; the colour of litmus whey changed into blue; no change in milk.

As it may be expected, that bacteria which occur under natural conditions in a blood containing medium would be able to decompose components of the blood, their action on a guinea-pig blood glucose agar has been tested. On this medium a distinct haemodigestion could be ascertained, *viz.*, by the occurrence of

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nutrient media at various temperatures: glucose agar, guinea-pig blood glucose agar, Besredka glucose agar in normal atmosphere and in an atmosphere containing carbon dioxide.

[1]) Gram positive cocci isolated out of the faeces from various bugs were examined along the same lines. Here as well the strains agreed mutually as far as their properties were studied. Fermentation of all sugars, coagulation of the milk and a changing into red of the litmus whey could be ascertained.

The bacteria isolated out of the vagina, which agreed so strongly in shape with the proventriculus bacteria, differed in all these features from the latter.

a green zone round the inoculation stroke. Further investigations into the blood affecting action were carried out in the Laboratory for Microbiology in Delft under the direction of Professor KLUYVER. We refer to our paper on „Affection of blood by bacteria" which will be published in this journal.

In so far as the investigation concerns the proventriculus bacterium isolated out of *Triatoma* we record the following: Already before the occurrence of any growth on the inoculation stroke on the blood agar plate a greenish discoloration some mm wide can be noted. After the lapse of one day the intensity of the oxyhaemoglobin bands in the spectroscope has considerably decreased in the zone of the green discoloration; the bands soon disappear completely. From the third day the intensity of the green colour decreases. In the literature such a green colour is frequently attributed to the presence of haematin, wrongly however. Presumably it is caused by the compound named „verdohaemochromogen" by LEMBERG (7) which forms the transition between haemoglobin and biliverdin. Besides the verdohaemochromogen which is formed as the result of the action of an exoenzyme produced by the bacteria, still other products of the breakdown of the haemoglobin are formed. The reaction on free iron indicates that at least part of the haemoglobin will change into iron free compounds (protoporphyrins).

Along with other features the fact, that gelatin is not liquefied by the proventriculus bacteria, indicates that this enzyme is not identical with the bacterial enzyme which liquefies gelatin, such as it has been stated repeatedly in literature.

Here follows the result of the preliminary investigation of this bacterium, which Professor KLUYVER had the courtesy to have carried out in his laboratory.

„An obligate aerobic, non-sporeforming rod, occurring also as diplo-rods, of very small dimensions, motile with cephalotrichic cilia (probably monotrichic). Gram negative, katalase positive, does not produce indole, does not reduce nitrates to nitrites (this has been tested in peptone water with 2 % KNO_3 in a test tube, Struyk bottle and stoppered bottle). Only in the test tube a very slight positive nitrite reaction could be noted, although in both kind of bottles the cultures did show development. Does not liquefy peptone gelatin, renders litmus milk alkaline (no further change in the milk), develops well at 20—37°C. (the bacteria were cultivated at 30° C. on the various media), does not ferment sugars (this was tested in peptone water 2 % glucose and yeast water 2 % glucose in Einhorn tubes and Struyk bottles; the bacteria did not develop in the closed arm of the Einhorn tube, in the Struyk bottle most abundantly in the pipe, further turbid and a bottom sediment), when tested with litmus and bromo thymol blue the bacteria form alkali.

The bacteria were kept on peptone agar. The colonies on peptone agar are of a pale cream colour and show frequently a peculiar

spreading; a star shaped zone occurring round the colonies. This zone shows a blueish iridiscence in transmitted light. The colonies are small. Strokes on slants show as well the peculiar lateral branching.

The same keeps true for slants with peptone gelatin with 12 % gelatin; this spreading, however, does not occur on slants with 4 % gelatin, where the culture is of a pale pink shade. Growth in peptone water abundant, turbid, a thick slime, pinkish sediment. In a stoppered bottle filled to the neck the bacteria did not develop. In a mineral medium with 0.4 % NH_4-succinate, 0.04 % K_2HPO_4, 0.02 % $MgSO_4$ the bacteria developed well; a distinct yellow green fluorescence of the medium such as in the case with *Pseudomonas fluorescens* and *Pseudomonas putida*, does not occur however. The medium in the long run takes on a dirty greenish colour.

These characteristics make it obvious that the bacteria belong to the genus *Pseudomonas*.

It has been tried to determine the bacterium by means of BERGEY's Manual 5th Edition. The description of *Pseudomonas cruciviae* Gray and Thornton agrees in some measure with the characteristics of the bacterium studied; for this species of *Pseudomonas* also a spreading of the colonies has been recorded.

A certain identification of the bacterium studied with the latter species, which probably has merely once been isolated from soil, can not be realised however."

At our question whether Professor KLUYVER knew this bacterium as an ubiquitous species, he answered in the negative, but this would by no means exclude the possibility, that this bacterium with its but slightly characteristic properties might be fairly widely distributed.

b. Agglutinogenic properties.

After it had been ascertained that blood serum of a rabbit did not show any spontaneous agglutination with a suspension of bacteria from one of the already mentioned four strains of proventriculus bacteria, this rabbit was immunized with the latter.

With the thus obtained serum agglutination reactions were performed with the strains isolated from various bugs. All strains were agglutinated to the limit of the titre (1/1600). This result can be deemed a further evidence for the reliability of the assumption that it is always a definite species of bacterium [1]) which occurs in the proventriculus and that the latter has been obtained in pure culture.

3. IDENTITY OF THE INTRACELLULAR AND LUMEN FORM OF THE PROVENTRICULUS BACTERIUM.

Still it remains a question whether the „lumen bacteria" are

[1]) This could not be checked in the pure culture of the proventriculus bacteria isolated from the faeces; here the assumed identity is thus based on morphological evidence.

identical with the bacteria which occur intracellularly in the wall of the proventriculus.

WIGGLESWORTH noted in his already referred to study of a series of sections that before the bug sucked blood bacteria are absent in the lumen of the proventriculus, but are present in the cells of its wall. If, however, the bug had sucked blood, he could note cells of the proventriculus, whose towards the lumen directed wall was damaged; along with this bacteria occurred in the lumen.

By a dynamic interpretation of these static data he concludes on an under natural conditions existing release of the intracellular bacteria into the lumen and thus on the identity of both forms. It is a matter of interest, however, that, when this identity actually proves to be true, the intracellular form apparently cannot be cultivated. An explanation might be looked for in the probably smaller number in which the intracellular form occurs compared with the number of bacteria present in the lumen, analogous cases existing in medical bacteriology. It must be left open, however, in how far this explanation is correct.

The following fact can be deemed at its most an indication, that this explanation for the fact that the intracellular form cannot be cultivated, is true. Out of six bacteria isolated at our request by Dr. S. J. C. DUNLOP with a micromanipulator from the proventriculus of an adult insect and inoculated on glucose agar, not a single one developed into a colony.

In quite another way we have tried to establish the identity of both forms, *e.g.*, by means of the following microscopic agglutination method. In some 500 young bug larvae which had not yet sucked blood and in which the bacteria would thus occur exclusively in the intracellular form the abdomina were removed. The remaining parts containing the proventriculus were ground in a mortar with sterile quartz sand. The pulp was suspended in a physiological salt solution and centrifuged at 3000 turns during 2 minutes. Previously it had been ascertained that at this speed and during this period bacteria, although on the verge of sedimentation, remained in suspension, whilst the coarser components of the tissue actually sedimented. The liquid remaining over the sediment and thus containing most of the bacteria was drawn into a pipette and centrifuged again, but now during half an hour at 9000 turns. Here as well a sediment was formed, but it appeared that in its upper layer the bacteria occurred in dense masses. This upper layer was accurately drawn up into a pipette and suspended in a small volume of the overstanding liquid. After 24 hours this suspension had again produced a sediment, whilst the overstanding liquid contained sufficient bacteria for the realization of microscopic agglutination.

In this investigation the following method of pipetting has been followed (see fig. 1): Unto a finely drawn out Pasteur pipette a rubber tube is fixed which ends into a rubber ball. By means of a clip the pipette is fixed to the tubes of a microscope, whilst the

rubber ball is held in a folded piece of cardboard between the stage and the object lens of another microscope. By means of the

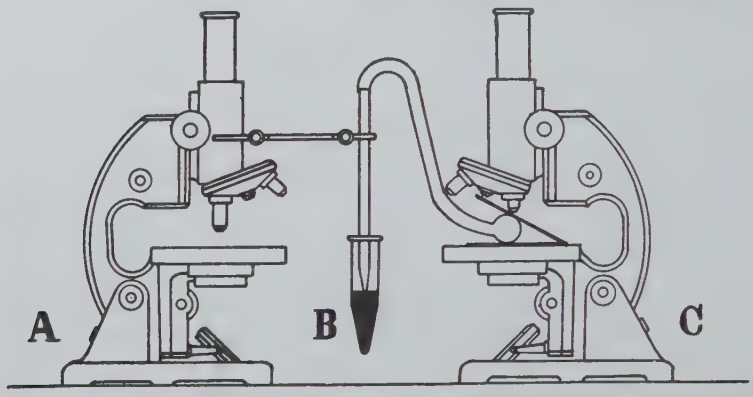

Fig. 1. A: Adjustment of the height of drawing in. B: Centrifugal tube. C: Adjustment of the rate of drawing in.

micrometer screws of both microscopes an accurate drawing in of the liquid may be realised. This method provides for a stable pipette, an accurate adjustment on the level of the liquid to be drawn up and an exact regulation of the rate of drawing in and the volume of the drawn in liquid.

The agglutination by means of an agglutinating serum at a dilution of 1/300 could be followed microscopically on a heated stage at 37°C. from beginning to end. A positive result was attained at; always after about an hour the agglutination was completely finished.

Hardly any doubt is left as to the identity of the intracellular and the lumen form of the bacterium.

4. The transmission of the bacteria on a new generation.

Wigglesworth (10) assumes that this transmission occurs by means of the egg, the infection occurring already in the ovary. When the larva is hatched the bacteria thus will occur already in the cells of the proventriculus. Further information as to the observations on which this assumption is based is not furnished in this paper.

As no direct communication exists between the proventriculus and the ovary, it has to be assumed that the bacteria would be set free from the cells of the proventriculus not only towards the lumen, but towards the haemocoele as well. They would have to make their way to the ovaries and penetrate into them. Doubtlessly a hard task for the bacteria!

It stands to reason that Buchner (3), who is not acquainted with any example of „ovary-infection" by intestinal, lumen- or epithelium symbiosis is of the opinion, that such an infection is

improbable in non-investigated cases as well. In a review of SCHWARTZ (9), however, some data are given which indicate that such an infection of the ovary might not be deemed an impossibility. So COWDRY (4) describes an infection in *Acarina*, which actually takes place in the way BUCHNER is doubtful of. In most of the like forms of symbiosis, however, an external infection takes place. In cases in which the symbionts occur in a mycetome in the haemocoele a transmission of non-motile symbionts in the haemocoele may sometimes be noted, notwithstanding the mycetome neighbours the ovary (experiments of KOCH (6) with *Lyctus linearis*). So it is not out of the question that the way of infection such as it was assumed by WIGGLESWORTH to occur in *Rhodnus prolixus* might occur similarly in *Triatoma infestans*. His assumption seems to be based mainly on two facts: *a*. The bacteria do not occur in the end-gut. *b*. Bacteria agreeing morphologically with proventriculus bacteria were noted in the ovaries.

In our investigations of *Triatoma infestans* the following facts could be ascertained:

The proventriculus bacteria may in fact occur in the end-gut of the rectum, although this is certainly not the usual case. At all events no letal agent occurs in the rectal fluid, as a culture of proventriculus bacteria was not perceptibly hampered when contents of the rectum, either non-sterilized or sterilized by heating, were dripped on the bacteria immediately after they had been streaked on the agar.

From the outer surface of the egg shell in two out of three cases in which it had been attempted at, cultures of the proventriculus bacterium could be obtained (agreeing morphologically and in cultural features).

A pure culture of proventriculus bacteria, streaked on a sterile glass plate, appeared to be able to stand a complete drying up during at least 8 days, without losing its viability in any way.

These data leave open the possibility of an external infection, such that the larva might infect itself during the hatching.

Still objections may be raised against this assumption: *a*. The larva has no biting mouth parts and it uses its proboscis only some time after hatching, its alimentary canal not having developed completely at that moment; this could be ascertained in sections of a newly hatched larva. So it is not clear how the bacteria might be transmitted from the surface of the egg shell into the intestinal tract. *b*. The occurrence in the egg of bacteria which agree morphologically with those from the proventriculus, although a culture of the former has not been attained at.

Both objections would lose their value, when it might be assumed that the bacteria pass through the egg shell into the interior of the egg. At present it is not possible to form an opinion as to the means by which this might occur. A passage through the micropyle might seem the most probable, although the place of the latter could not be determined in a preliminary microscopic examination, either

in eggs which had left the mother bug in the normal way, or in those which, already provided with a complete shell, had not yet reached the vagina and had thus not yet been fecundated. Nor was an attempt successful to determine the micropyle by means of artificial fecundation, as we did not succeed in the finding of a medium in which the spermatozoa would be stimulated to motility.

In this connection we point to the occurrence of proventriculus bacteria (ascertained culturally) in the liquid of the penis, and not mentioned as yet, in the receptacula seminis (ascertained microscopically).

Summary.

1. From the proventriculus of *Triatoma infestans* a *Pseudomonas* species could always be obtained in pure culture by simple means.
2. The bacteria isolated out of the ventriculus of various bugs appeared to agree, not only morphologically but also biochemically and agglutinatorically.
3. Further evidence for the identity of the intracellular and the lumen form of this bacterium could be furnished by serological means.
4. The assumption expressed by WIGGLESWORTH that the infection of the young bug would occur already in the egg and his view on the means by which the egg is infected are very much open to question.
5. It does not appear probable that the bacterium, isolated by WIGGLESWORTH from *Rhodnus prolixus* is identical with the proventriculus bacterium of *Triatoma infestans*.
6. The intracellular bacteria set free into the lumen of the proventriculus have in virtue of their haemodigestive capacity, probably a function in the digestion of the blood sucked by the bug.

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IMMUNITY IN RABBIT-PLAGUE IMMUNOLOGICAL RELATIONSHIP WITH COW-POX

by

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1. Introduction.

In 1941 we described an acute morbidity in rabbits, that was caused by a filterable virus. In the course of the illness, which lasted only a few days, the owner of the animals had observed no other symptoms than listlessness, and somewhat too soft fecal discharges. The virus was demonstrable in the bile, blood, urine and nasal exudate. Rabbits can be infected by subcutaneous, intracutaneous, intranasal, conjunctival and intravenous infection, and also by infection per os. Experiments showed that the disease is easily transmissible by contact. Experimentally infected animals usually die. At the point of infection an extensive oedema almost always arises. The details have been described in previous communications on these subjects (1, 2, 3, 4, 5, 6). These publications also report on the pathogenicity of rabbit plague virus for other species, and on the cultivation of the virus on the chorio-allantois of the chicken egg. Our later experiments deal principally with the immunity acquired through infection with the virus. As it proved possible to infect guinea-pigs by rubbing the virus into the scarified footsole, a mode of infection which causes a pock-like reaction, we made experiments in cross-immunity with pox viruses. A report on these experiments and others of minor importance follows below.

2. The immunity to rabbit-plague caused by infection with the rabbit-plague virus.

A. in rabbits.

Exp. 1. Immunity after injection of virus stored for a long time.

On 9-9-41 rabbits 339 and 340 were injected subcutaneously with 0.75 cc of a suspension of material taken from rabbit 317, that had died of rabbit-plague.

This material had been preserved for 84 days at —20° C. in glycerol 80 %. Rabbit 339 died of rabbit-plague after 8 days.

Rabbit 340 was listless during a few days, showed loss of appetite, but soon recovered. Some time later (22-2-42) it was proved that this rabbit could be placed in infected cages without becoming ill, and that it was not susceptible to conjunctival or subcutaneous injections. The virus administered in the first experiment was of just the right dose and virulence to cause immunity but was not lethal. Conclusion: Virus stored at —20° C. can induce immunity.

Exp. 2. The immunity caused by injection of a highly diluted virus-suspension.

In order to determine the M.L.D. of the liquid from the subcutis oedema of a rabbit, a number of rabbits were injected with dilutions of this material. On 23-10-41 rabbit 358 was injected subcutaneously with 0.0000005 cc. This rabbit, that had received the least virus, was the only animal that showed no symptoms and proved to be completely immune to reinfection on 12-2-42. Conclusion: A very small dose of virulent virus confers good immunity.

Exp. 3. The immunity caused by injection of filtered virus.

On 3-3-42 rabbit 377 was injected with a Berkefeld N filtrate of material taken from rabbit 376. A total amount of 1 cc was injected intracutaneously and subcutaneously into the back of the rabbit. On 10-3-42, the site of injection is red and thickened. On 11-3-42, this reaction has greatly increased, the center is livid; thereafter the reaction decreases slowly. The animal's general condition was always normal. On 19-3-42, the colour of the skin is again almost normal. The site of infection is still somewhat inflexible. The animal was then infected on the conjunctivae with virulent virus, *viz.*, unfiltered and undiluted liquid from a subcutis oedema. The animal remained entirely healthy, but a reaction was visible on the site of the former infection on the back. This again became red and thick. During the next few days, some necrotic tissue was expelled from the center. Thereafter the lesion healed completely. The animal proved to be insusceptible to a subcutaneous infection on 15-4-42. This shows that after injection of filtered material immunity developed within 16 days.

Exp. 4. Immunity after recovery from the disease.

On 2-3-42 rabbit 372 was infected on the conjunctivae of both eyes with one drop of unfiltered material from an oedema of rabbit 376. The animal became seriously ill, but recovered. Rabbit 394, infected in one eye with the same material in the same way, showed the same reaction. Both animals were insusceptible to subcutaneous injection of virulent virus on 15-4-41. Conclusion: On recovery from the disease after conjunctival infection, immunity is present.

Exp. 5. Immunity after a contact infection that caused no symptoms.

On 11-9-43, rabbit 502 was placed in one cage with rabbit 499. Rabbit 499 had been infected subcutaneously on 3-9-43, and died ten days later of rabbit-plague. Rabbit 502 was left alone in the infected cage. In contrast to other rabbits, which died after a contact infection, this rabbit remained clinically normal. Some time

after, on 23-10-43, this rabbit was unsusceptible to a subcutaneous injection of a dose of virus that killed the control rabbit in 8 days.

Although rabbit 502 became infected with virus from rabbit 499, this virus gave rise to immunity without causing the disease. Some time after rabbit 502 gave birth to a litter of seven. On 5-8-44, when the young rabbits were 2 months old, they and their mother were injected subcutaneously with rabbit-plague virus. All the young rabbits died after a week. The mother animal remained normal.

The control rabbits used in these 5 experiments all died of rabbit-plague. The immunity was further proved by the demonstration of virus-neutralizing antibodies in the serum.

Exp. 6. The neutralisation of virus with immune-serum collected from rabbits.

1. Experiments with mice.

Serum was collected from two rabbits, 340 and 358, which had been proved to be immune, and also from a normal rabbit. The serum from the two immune rabbits was mixed. To 1 cc immune-serum and to 1 cc normal serum, 0.3 cc of a suspension of mouse-brains were added.

These brains were taken from mice which had died after intracerebral infection with rabbit-plague virus (27th mouse-brain passage). 0.5 gram of brain was suspended in 2 cc physiological NaCl solution. After inoculation with the virus, both tubes of serum were incubated for 5 hours in the dark at 20° C. Then 3 mice were injected intracerebrally with each mixture. The 3 mice injected with the normal serum-virus-mixture all died after 4 days. This is the normal period. Of the other 3 mice, 2 remained normal, 1 died after 8 days. This proves that the serum-mixture of rabbits 340 and 358 contained specific antibodies. The fact that rabbit 402 died after subcutaneous injection with 0.5 cc of the immune-serum-virus mixture proves that the virus was not totally inactivated. However this rabbit became ill 3 days after the control rabbit.

2. Experiments with rabbits.

The antibodies could also be demonstrated by experiments with rabbits. Rabbit 403 was injected subcutaneously on the left leg with a very large dose of virulent virus (0.1 cc fresh, undiluted oedema liquid). At the same time 6 cc serum mixture from rabbits 340 and 358 was injected intravenously and also subcutaneously in the right leg. The animal showed no symptoms. This proves the presence of antibodies in the immune serum. This rabbit, which had been simultaneously injected with the virus and the immune-serum, later proved to be insusceptible to an infection with virulent virus.

B. in mice.

Exp. 7. Experiments on the intracerebral infection of mice have been previously published (5, 6). At that time the 39th passage

had been reached and the virus was still unchanged in its pathogenicity for rabbits. These experiments were continued up to the 72nd passage. On 6-4-43, rabbit 482 was infected in one eye with brain suspension of the 72nd mouse-brain passage.

On 11-4-43 conjunctivitis and swelling of the eyelids could be observed. The animal died on 14-4-43, which is the normal course of the disease (the post-mortem showed subcutis oedema, liver-foci, and petechial haemorrhages in the lungs).

It was shown that mice, infected subcutaneously with virulent rabbit-plague virus remained clinically sound. However, they have become insusceptible to an intracerebral injection, which is lethal for normal mice. In mice as in rabbits, the rabbit-plague virus (R-P), causes immunity to the disease. This was also shown in the following way. The intracerebral infection with R-P is almost always mortal. The mice used for the first 47 passages all died of R-P. The 48th passage mouse did not die. This mouse had been infected on 13-1-42 in exactly the same way as the mice used for the 44th, 45th, 46th and 47th passage. On 7-2-42, this mouse was reinfected intracerebrally with brain material of the 15th mouse passage. Ten other mice were infected in the same way with this material. These latter mice all died, but the mouse that had been injected twice did not. The first injection on 13-1-42 had rendered it immune.

C. in guinea-pigs.

Exp. 8. Some infection experiments with a negative result, at first led to the conclusion that guinea-pigs could not be used for work on rabbit-plague virus. Formerly it was also thought that guinea-pigs were insusceptible to foot-and-mouth disease, until it was found that the infection can be carried out by applying the material to scarifications on the foot-sole. This method of infection is also always successful in the case of R-P. The method is entirely the same as is customary with foot-and-mouth disease. The foot-soles are first cleansed with a wad of cotton-wool dipped in physiological salt solution, then they are dried with dry cotton-wool. Three scarifications are made with a vaccinostyl, if possible in such a way that almost no blood is drawn. The virus-containing material is then rubbed into the scarifications. A pock-like vesicle develops, containing pus. As this pock-like reaction was the result of the first passage in a guinea-pig, we tried to ascertain whether the virus could be made dermotropic through repeated guinea-pig passages, and so cause the same pocklike reaction in the rabbit. The following passages were made on the foot-soles of guinea-pigs:

1943	7—5	1. passage	Guinea-pig	497 and 498
	12—5	2. ,,	,,	499 and 500
	18—5	3. ,,	,,	501
	24—5	4. ,,	,,	488 † 21—7
	31—5	5. ,,	,,	513 † 22—6

4—6	6. passage	Guinea-pig.	514	
8—6	7. ,,	,,	519	
12—6	8. ,,	,,	520	
17—6	9. ,,	,,	521	
25—6	10. ,,	,,	527	
30—6	11. ,,	,,	534	
8—7	12. ,,	,,	535	
14—7	13. ,,	,,	538	and 539; rabbit 494 and 495
17—7	14. ,,	,,	541	
20—7	15. ,,	,,	542	and hen 350
26—7	16. ,,	,,	543	

On 14-7-43, material from the 12th guinea-pig passage was used to infect guinea-pigs 538 and 539 on the foot-soles, rabbit 494 cutaneously and rabbit 495 subcutaneously. Rabbit 494 was infected as follows: the skin was first shaved, then 4 small scarifications were made, in which the material was rubbed. Three days after the infection both guinea-pigs had a positive reaction. The four scarifications of rabbit 494 were surrounded by a red, oedematous zone which was 0.75 cm in diameter. Because of the surrounding swelling, the scarifications gave the impression of lying deeper in the skin. The subcutaneous injection of rabbit 495 resulted in an oedema at the point of injection, on the hind leg. This oedema spread on both sides of the Achilles tendon and the heel. On 19-7-43, the site of injection of rabbit 494 was somewhat swollen and lived. The reaction is in no way pock-like. On 22-9-43, the animal succumbed. The post-portem examination brought to light changes typical for R-P. Rabbit 495 had died the day before. Conclusion: After the R-P virus has been passed through guinea-pig epithelium twelve times, it is unchanged in that it still causes a septicaemia in the rabbit.

In contrast to pox in guinea-pigs, no pox ever developed elsewhere on the body, after the primary eruption at the site of injection. A few of the guinea-pigs infected with R-P die. Sometimes macroscopic and microscopic changes are found which should be considered as caused by the plague virus. Most guinea-pigs recovered from the primary vesicle; when these animals are later infected on the same or on an other foot-sole, no reaction ensues, so that immunity must have resulted from the first infection.

These experiments lead to the conclusion that it is possible to immunize rabbits, mice, and guinea-pigs against R-P with R-P virus.

In the following experiments this immunity is also obvious from the reactions of the control animals.

3. Cross-immunization experiments with R-P and vaccin virus in guinea-pigs.

In this connection I wish first to describe an experiment which was planned to give us an opinion as to the immunity to R-P virus

caused by the infection of guinea-pigs with R-P virus, but which also supplied us with some data on the relationship between the R-P and cow-pox viruses. Table I gives a survey of this experiment.

From the experiments in Table I it is clear that a positive R-P reaction on a foot-sole completely immunizes a guinea-pig against a second infection with R-P (see guinea-pigs 316, 326, 328, 329). Guinea-pig 314, immunized in the testicle, proved to be the only other completely immune animal. In almost all the other guinea-pigs, immunity was only partial. But one animal, number 308 was still completely susceptible. It was also found that cow-pox immunizes against R-P, especially if the cow-pox virus has caused a lesion on the foot-sole. On 18-1-43, the guinea-pigs 375, 376, 305, 306, 307, 308, 311, 312, 313, 314, 316, 326, 328 and 329 were infected on a foot-sole with cow-pox. None of these animals, that had all been infected once or twice with R-P, reacted. The reaction of the control animals 421 and 422 was distinctly positive. Therefore it is possible to conclude that R-P infection renders guinea-pigs immune to cow-pox.

In order to compare the immunity to R-P and to cow-pox caused by R-P virus more easily, the following experiment was made (Table II).

This experiment again proves that R-P virus immunizes against the disease. Guinea-pigs 384 and 385 were already completely immune after one month. The corresponding guinea-pigs of the R-P-cow-pox experiment were all completely immune to cow-pox, only the most recently immunized guinea-pig 388 was but partially immune. Both experiments prove that one R-P vesicle immunizes against R-P and against cow-pox.

Further experiments (see Table III) lead to the conclusion that the immunity to cow-pox caused by R-P, decreases after a time (Compare the group infected in March with the other group).

In this experiment the R-P immunity usually could not prevent a local cow-pox reaction; the reverse, complete immunity to R-P through cow-pox infection, was always true, as is shown in Table IV.

Tables V and VI give the results of two experiments, each composed of two parts, in which the cross-immunity is considered.

In connection with the results given in Table V it is pointed out that the guinea-pigs previously inoculated with R-P were immune to cow-pox. This is clear, in the first place because the two control animals became seriously ill from a generalized pox infection, while the entire R-P group had no symptoms but a vesicle on the site of infection. This developed more slowly than the local reaction of the controls, which was also more serious. There was a great difference in the time elapsing before the animals had recovered; the entire R-P group had recovered, while the control animals were still seriously ill from the local lesion and the generalized disease. The period of time elapsing between the

Table I.

Guinea-pig:	1st infection	reaction	10-12-42 on foot-sole of both hind feet R—P	reaction 14-12-42	reaction 17-12-42	reaction 21-12-42
302	R–P intradermal 16-9-42	+ ?	„	+ +	* *	healing
305	„ cutaneous „	?	„	+ +	* *	healing
306	„ „ „	?	„	+ +	* *	healing
307	„ conjunctival „	—	„	+ +	* *	healing
308	„ „ „	—	„	+ +	+ +	+ +
311	„ intravenous „	—	„	+? +?	* *	healing
312	„ „ „	—	„	+ +	* *	healing
313	„ intratesticularly „	—	„	+? +?	* *	healing
314	„ „ „	—	„	— —	— —	— —
316	„ on the foot-sole „	+	„	— —	— —	— —
326	„ „ 14-9-42	+	„	— —	— —	— —
328	„ „ „	+	„	— —	— —	— —
329	„ „ „	+	„	— —	— —	— —
317	cow-pox on the foot-sole	+	„	— —	— —	— —
319	„ cutaneous	+	„	+ +	* *	healing
320	„ intradermal	?	„	+ ??	* *	healing
375	control		„	+ +	+ +	+ +
376	„		„	+ +	+ +	+ +
377	„		„	+ +	+ +	+ +
378	„		„	+ +	+ +	+ +

The intradermal and cutaneous infections were carried out after removing the hair from the skin.

? = doubtful reaction; +? = slightly positive reaction; + = distinctly positive reaction; * = an atypical reaction. This reaction was slighter than +, and characterized by a peripheric red reaction zone; all animals with this reaction recovered unusually quickly. Two symbols are given in the last three columns, one for each hind-foot.

Table II.

R-P — R-P					R-P — Cow-pox					
Guinea-pig	R-P infection	reaction	15-1-43 R-P infection	reaction 18-1-43	Guinea-pig	R-P infection	reaction	18-1-43 cow-pox infection	reaction 20-1-43	reaction 25-1-43
366	on l. and r. hind foot 3-12-42	+	l.	—	368	as 366	+	l.	—	—
367	„	+	l.	—	369	as 367	+	l.	—	—
375	on l. and r. hind-foot 10-12-42	+	r.	—	376	as 375	+	l.	—	—
378	„	+	l.	—	377	as 378	+	l.	—	—
384	on l. hind-foot 16-12-42	+	r.	—	388	as 384	+	r.	+?	scaling healing
385	„	+	r.	—	389	as 385	+	r.	—	
415	control		l.	+	421	control		l.	—	+ large vesicle containing pus
416	„		l.	+	422	„		l.	—	ditto

Table III.

Guinea-pig	date of R-P infection	material used for R-P infection	cow-pox 22-9-43 on left hind-foot*	27-9-43	30-9-43	4-10-43
465	5-3-43	66 mouse passage		+	+	+
323	9-3-43	1 guinea-pig passage		—	+	r
324	„	„		+	+	r
325	„	„		+	+	r
466	„	„		+ +	+ +	r
467	„	„		+ +	+ +	r
469	„	„		+ +	+ +	r
472	„	„		+ +	+ +	r
473	„	„		+	+ +	r
474	„	„		+	+ +	r
475	„	„		+ +	+ +	r
497	7-5-43	oedema-liquid of a rabbit		—	(+)	R
499	11-5-43	2 guinea-pig passage		(+)	(+)	r
500	11-5-43	2 „		—	(+)	R
501	18-5-43	3 „		+	(+)	R
514	4-6-43	6 „		?	+	R
519	8-6-43	7 „		+	+ +	r
520	12-6-43	8 „		+ +	+ +	R
521	17-6-43	9 „		+ +	+ +	r
527	25-6-43	10 „		?	—	R
534	30-6-43	11 „		+	(+)	R
535	8-7-43	12 „		—	+	R
538	14-7-43	13 „		—	+	R
541	17-7-43	14 „		+	+ +	r
542	20-7-43	15 „		—	—	—
549	control			+ +	+ +	+ +

— = negative; ? = doubtful; (+) = slightly positive; + = positive; + + = distinctly positive; r = recovering; R = recovered.

* all guinea-pigs were infected with fresh cow-pox material taken from three guinea-pigs that had been infected with virus from a case of spontaneous cow-pox in a calf (Rijnauwen strain).

Table IV.

Guinea-pig	4-2-43 infected on left hind-foot with cow-pox	13-2-43	22-2-43	9-3-43 infected on right hind-foot with R-P	16-3-43
444	„	+	G	„	—
445	„	+	G	„	—
446	„	+	G	„	—
447	„	+	G	„	—
448	„	+	G	„	—
449	„	+	G	„	—
450	„	+	G	„	—
451	„	+	+	„	—
452	„	+	†		
453	„	+	G	„	—
466	control			„	+
467	„			„	+
468	„			„	+
469	„			„	+
470	„			„	+
471	„			„	+
472	„			„	+
473	„			„	+
474	„			„	+
475	„			„	+
323	12-1-43 pulled through a R-P reaction			„	—
324		„		„	—
325		„		„	—
417	13-1-43 pulled through a cow-pox reaction			„	—
418		„		„	—
421	18-1-43	„		„	—
422		„		„	—

— = negative; + = positive; G = generalisation; † = died.

inoculation and the infection of the guinea-pigs, was the shortest in the case of guinea-pig 595. This animal had the slightest local reaction and recovered first.

Guinea-pig 621, inoculated with cow-pox, was entirely immune. The immunity of this guinea-pig was therefore more complete than that of the R-P group [1]).

[1]) The history of guinea-pig 604, that was added to the experiment, was as follows: On 2-11-43 guinea-pigs 604 and 605 were infected with cow-pox on the foot-sole and placed together in one cage. Number 605 died of pox, 604 remained clinically healthy, but from the reaction after the second infection it is permissible to conclude that this animal had been rendered partially immune.

Table V.

R-P — cow-pox immunity and
Cow-pox — cow-pox immunity in guinea-pigs

Guinea-pig	left and right hind-foot infected with R-P	reaction	27-2-44 left hind-foot infected with cow-pox	4-3-44	6-3-44	14-3-44
575	2-11-43	+	„	+	+	recovered
577	8-11-43	+	„	+	+	„
578	12-11-43	+	„	+	+	„
583	23-11-43	+	„	+	+	„
587	1-12-43	+	„	+	+	„
595	18-12-43	+	„	+	+	„
598	control		„	+ +	+ +G	G ill
599	„		„	+ +	+ +G	G ill

Guinea-pig	infected with cow-pox	reaction	27-2-44 left hind-foot infected with cow-pox	4-3-44	6-3-44	14-3-44
621	2-11-43	+	„	—	—	—
623	control		„	+ +	+ +G	G ill
626	„		„	+ +	+ +	G ill
627	„		„	+ +	+ +	G ill
710	„		„	+ +	+ +G	G ill
604	2-11-43	—	„	+	re-covering	re-covered

— = no reaction; + = positive local reaction; ++ = distinctly positive local reaction; G = generalisation.

The only difference between the experiments described in Tables V and VI is that in the experiments described in Table VI the animals were reinfected with R-P. In this latter experiment both groups were found to be totally immune to R-P. All controls, however, reacted. In order to get a more exact conception of the cross-immunity, these experiments were all made with guinea-pigs of the same stock; all were non-pregnant, female animals of the same weight. They were all fed with the same food and were kept in the same stable. The R-P virus, used in these experiments, was always very pathogenic for rabbits. The cow-pox virus was also very virulent. It was originally taken from a spontaneous case

Table VI.

Guinea-pig	left and right hind-foot infected with R-P	reaction	27-2-44 left hind-foot infected with R-P	4-3-44	6-3-44	14-3-44
572	28-10-43	+	„	—	—	—
574	2-11-43	+	„	—	—	—
576	8-11-43	+	„	—	—	—
582	23-11-43	+	„	—	—	—
589	7-12-43	+	„	—	—	—
594	18-12-43	+	„	—	—	—
596	control		„	+	+	recovering
597	control		„	+	+	recovering

Guinea-pig	left and right hind-foot infected with cow-pox	reaction	27-2-44 left hind-foot infected with R-P	4-3-44	6-3-44	14-3-44
602	28-10-43	+	„	—	—	—
607	8-11-43	+	„	—	—	—
620	13-12-43	+	„	—	—	—
622	control		„	+	+	recovering
624	„		„	+	+	recovering
628	„		„	+	+	recovering
629	„		„	+	+	recovering

of cow-pox in a calf, and subsequently passed through guinea-pigs.

The experiments show that cross-immunity exists. The immunity caused by cow-pox for both R-P and cow-pox is total, while R-P protects totally against R-P but only partially against cow-pox. The action of these viruses in guinea-pigs is not identical. In the first place, there is a difference in the local reaction: the R-P lesion is less extensive than the cow-pox lesion. The contents of the latter are more pus-like, while the subepithelial layers of the sole are more affected and the foot is much more swollen. In the second place, virulent cow-pox in the guinea-pig is always followed by generalisation, for instance to the area surrounding the anus and the vulva, the nose and the mouth. In the case of R-P virus, this spreading was never noticed, even after 16 passages through the guinea-pig. We may say therefore that the guinea-pig reacts differently to infections with R-P and cow-pox; reinfection with

cow-pox proves that these two viruses also give two different kinds of immunity; they are therefore related but not identical. An infection with R-P virus does not even protect entirely against a not very virulent strain of cow-pox virus, for example a strain that gives only a local reaction in the guinea-pig. This was the case with guinea-pig 388 (see Table II). In order to consider this point more closely, the immunity of a group of guinea-pigs that had been infected on both hind feet with R-P and again some time later with the same virus, and the immunity of a group infected once on one foot-sole with R-P, were tested against a moderately virulent cow-pox strain. The results are given in Table VII.

This cow-pox strain caused a distinctly positive reaction in the control animals 741 and 742, the inoculated foot-sole showed a large pock-vesicle containing pus, the foot was extremely swollen, but generalisation did not occur. The R-P group is clearly immune. Nine animals did not even react locally, eight reacted but slightly; but this slight reaction again proved that the R-P and cow-pox viruses are not identical although related.

4. Immunity experiments in cattle.

On 16-9-42, calf 714 was infected intradermally, cutaneously and conjunctivally with R-P. The material used was unfiltered liquid from an oedema and from the organs of rabbit 447. This rabbit died of R-P. The conjunctiva showed no reaction, the intradermal and cutaneous reactions were slight and indistinct. The control rabbit 452 died on 23-9-42. On 25-1-43, this calf was infected by scarifying the hide and rubbing the scarifications with material collected from cows that had suffered from pox. Since 1938, a part of this material had been kept in 50 % glycerol and another part as a dry powder in vacuum at —20° C. As a control, calf 715 was infected with the same material. On 2-2-43 slight reaction was perceptible in calf 714. Two days later some round, dry, crusty, elevated spots had developed.

On this date, 4-2-43, a number of round, rather dry spots were noted in calf 715. This reaction had not been observed earlier, and was considered to be positive, though atypical. The reaction of calf 715 was much more distinct than that of calf 714. On 8-2-43 the reaction of 714 was decreasing, while that of 715 was still very distinct. On 9-2-43 the reaction of 714 is only just perceptible, while that of 715 is still very distinct.

Although an experiment with one animal can not lead to a definite conclusion, it gave us the impression that the calf that had been inoculated with R-P, was at least partially immune to cow-pox. This agrees with the results of the experiments with guinea-pigs.

Material taken from calf 715 was used to infect calf 716 on 4-2-43. This animal developed a distinct lesion at the site of inoculation. Some time later, calves 715 and 716 were infected

Immunity expe

Guinea-pig	left and right hind-foot infected with R-P	reaction	27-2-44 one foot infected with R-P	reaction	18 one inf with
572	28-10-43	+	left	—	ri
574	2-11-43	+	„	—	
576	8-11-43	+	„	—	
582	23-11-43	+	„	—	
589	7-12-43	+	„	—	
594	18-12-43	+	„	—	
698	16- 2-44	+	„	—	
699	16- 2-44	+	„	—	
700	16- 2-44	+	„	—	
704	22- 2-44	+	„	—	
705	22- 2-44	+	„	—	
622			„	+	
624			„	+	
709			„	+	l
717			„	+	r
596			„	+	l
597			„	+	r
741	control				l
742	„				l

— = negative; ? = doubtful; (+) = slightly positive; + = distinctly positiv

intracutaneously at the same time on two different parts of the hide with R-P and cow-pox. Calf 719 was infected in the same way as a control. The material, in the case of both viruses, was powdered, dried virus, suspended in 50 % glycerol. As a further control, hen 490 was infected with the R-P virus and hen 491 with the cow-pox virus. The results are summarized in Table VIII.

As is shown in Table VIII, both calves inoculated with cow-pox were immune to cow-pox and R-P, although a slight local reaction was perceptible. The control calf had a positive reaction, which was however, not as distinct as might have been expected. The reaction observed in hen 491, although clearly positive, was less

R-P — cow-pox

ion -44	22-3-44	27-3-44	31-3-44	conclusion
	—	—	—	—
	—	—	—	—
	—	—	—	—
	—	skin some what loose and dry	skin peeling off	?
	—	—	—	—
	some moisture, swollen	skin some what loose and dry	skin peeling off	(+)
	—	—	—	—
	—	some pus	healing	(+)
	—	—	—	—
	—	—	—	—
	—	—	—	—
sture at of in-	some pus (healing)	healing	skin peeling off	(+)
	skin somewhat loose and dry	skin peeling off	skin peeling off	?
	—	—	—	—
	—	very slight reaction	healing	?
	—	—	skin slightly scaling off	?
	—	—	skin slightly scaling off	?
	+	+	+	+
	+	+	+	+

distinct than the reaction usually obtained in hens with cow-pox. The original, dried virus of 1938 was used for the infection, as we had no calves at our disposal in which to pass the virus and so acquire fresh material. Perhaps a repetition of this experiment with more animals and virulent strains of the viruses would have shown differences in the immunity caused by the two viruses, but owing to the war this was not possible.

A more detailed account of the infection of chickens and other birds will follow. At all events the immunity of hen 466 points to the relationship of R-P and cow-pox.

Table VIII.

	First infection	reaction	11-5-44 infected with:	14-5-44	17-5-44	20-5-44	22-5-44	24-5-44	conclusion
Calf 715	**25-1-43** with cow-pox	+	cow-pox	—	—	—	—(?)	—(?)	immunity
			R-P	—	? some small spots not clearly defined	slightly + but much less than 719	—(?)	—(?)	immunity
Calf 716	4-2-43 with cow-pox	++	cow-pox	—	—	—	—(?)	—(?)	immunity
			R-P	—	—	—	—	—	immunity
Calf 719	control		cow-pox	—	+? (swollen slight eruptions)	+	+ reaction increased	++	
			R-P	—	+ distinct round spots	+	+ crusts fall off	+ healing	
Chicken 466	5-4-44 with R-P	++	cow-pox	—	—	—	—	—	immunity
Chicken 490	control		R-P	+	++	++ decreasing			
Chicken 491	control		cow-pox	—	+	+ decreasing			

5. Immunity experiments with rabbits.

Rabbits 340 and 358 were immunized with R-P virus and then infected by rubbing cow-pox virus into scarifications of the skin. The control rabbits 381 and 398 were infected in the same way. The cow-pox strain had been obtained from the cow-pox Institute in Amsterdam. The reactions that developed were not distinct but the impression was received that the rabbits 340 and 358 also reacted slightly. This experiment was repeated in the following way: 4 R-P-immune rabbits 372, 377, 394, 403, and three control rabbits were used. The abdomen of each of these animals was shaved. Two days later, the four immune rabbits and the control rabbits 405 and 407 were infected by rubbing the cow-pox strain from Amsterdam into punctures made in the skin with a vaccinostyl. These two control rabbits received three additional punctures which were not infected. The results are shown in Table IX.

Although none of the lesions were very typical, we still could conclude that both the immunized and the non-immunized rabbits reacted to the cow-pox virus; no immunity was noticeable. In guinea-pigs the immunity to cow-pox caused by R-P inoculation was almost always distinctly visible, but less so than the immunity to R-P caused by cow-pox. Other experiments, which will be described further on, made the impression that this difference in immunity between the two viruses is even stronger in rabbits. Rabbits 405 and 407, infected with cow-pox virus as control in experiment IX proved to be immune to subcutaneous infection with R-P on 11-6-42. This was also true of rabbits 442 and 443, that had been infected cutaneously with strain Amsterdam on 21-8-42 and reinfected with R-P on 9-9-42 (The control rabbits 446 and 447 both died on 8-9-42). Even when the cow-pox lesion in the rabbit is very slight, immunity is practically complete. For instance, rabbit 451 was infected on 16-9-42 with cow-pox (strain Amsterdam); the lesion was very slight. On 12-1-43, this rabbit was injected subcutaneously with $\frac{1}{2}$ cc brain suspension of the 58th mouse brain passage of the virus, but remained healthy. Only on the site of injection a swelling developed, which was followed by necrosis. The control rabbit, that had been infected with the same material, developed an enormous oedema on the site of infection, and died. Rabbit 358 that was immune to R-P was also infected with the same material but remained healthy and had no lesion at the point of injection. This proves that the local lesion of rabbit 451 was caused by the R-P virus and not by the brain tissue also present in the suspension. Rabbit 496, that had developed so slight a lesion that the reaction was judged to be almost negative, proved some time later to be immune to infection with R-P. This rabbit had been infected cutaneously on 16-7-43, with a 5th tissue culture passage of a cow-pox strain (Rijnauwen). Nine months later, on 5-4-44, the animal was injected subcutaneously with 0.75 cc fresh material of a rabbit that had died

Table IX.

Rabbit no	recovered from R-P	infected with cow-pox 20-5-43	not infected punctures	reaction 22-5-43	reaction 23-5-43	reaction 26-5-43	reaction 29-5-43
372	„	3 punctures		3 (+)	3 +	+	decreasing
377	„	3 „		3 (+)	2 + ; 1 (+)	+	„
394	„	3 „		3 (+)	3 +	+	„
403	„	4 „		3 (+)	4 +	+	„
405		3 „		3 (+)	3 +	+	„
407		3 „		3 (+)	1 + ; 2 (+)	+	„
405			3 punctures	—	—	—	—
407			3 „	—	—	—	—
406			3 „	—	—	—	—

of R-P; it remained healthy. This dose may be considered to be more than one hundred thousand times the M.L.D. The immunological relationship of R-P and cow-pox has therefore also been proved by these experiments with rabbits, but again the viruses did not seem to be identical. The immunity to R-P caused by cow-pox is so complete, that the problem of prophylactic measures against this disease is solved. R-P seems to occur but sporadically. Vaccination of animals in danger of infection will undoubtedly check the disease at once. Only one vaccinated rabbit died of R-P. This occurred in the following experiment. On 10-10-43, the rabbits 504, 503, 487, and 489 were injected subcutaneously with R-P virus. Rabbit 503 had been vaccinated on 17-10-43 with cow-pox strain Rijnauwen. This strain had first been passed 8 times through chicken-eggs and 5 times through tissue cultures. The reaction was positive. Rabbit 504 was a control animal. Rabbit 487 had been vaccinated on 8-6-43 with the cow-pox strain Rijnauwen, which had now been passed first 4 times through chicken-eggs and then once through a hen. The reaction was uncertain. Rabbit 489 had been vaccinated on 25-6-43 with strain Rijnauwen after 5 chicken-eggs passages; the reaction was positive. After the injection of R-P virus, rabbits 487 and 489 remained normal, control rabbit 504 died of R-P, but so did rabbit 503. Until the immunity resulting from virus cultivated in tissue-cultures and eggs has been compared to that resulting from virus collected directly from animals, it is perhaps better to use only the latter source in immunity experiments.

5. Immunity experiments in birds.

As soon as the experiments described above had brought to light a possible relationship between R-P and cow-pox, a number of chickens were infected intrafollicularly, on the thigh, after about 15 feathers had been pulled out. The animals reacted very distinctly with oedema of the skin and a swelling of the feather follicles.

In Table VIII mention was made of hen 466, that had been immunized against cow-pox with a R-P infection (The control hen 491 reacted to the cow-pox virus.) The reverse, immunity to R-P after vaccination with cow-pox, had already been demonstrated in a hen. While these experiments were being made, a turkey-pox virus was isolated from turkeys. For the experiments on this virus, which will be described in an other publication, birds were used. These birds and some chickens that had been inoculated with cow-pox, were examined for a possible immunity to R-P. The results are given in Table X.

Table X shows that birds can be successfully used for experiments on R-P, if the intrafollicular method of infection is used. The relationship between R-P and cow-pox is again demonstrated, as the entire group of chickens that had previously been infected with cow-pox was immune. On the other hand, all birds inoculated

Table X.

		First infection	reaction	5-4-44 infected intrafollicularly with R-P*)	reaction
Cock	901	cow-pox 27-8-43 on one leg with dried virus (strain Rijnauwen)	+	left thigh	—
Chicken	902	„	+	„ „	—
„	903	„	+	„ „	—
„	904	„	+	„ „	—
„	905	„	+	„ „	—
„	906	„	+	„ „	—
„	907	„	+	„ „	—
„	908	„	+	„ „	—
„	909	„	+	„ „	—
„	910	„	+	„ „	—
Chicken	968	left T 5-10-43: + ; right P 3-11-43	+	„ „	+ (swelling of the follicles)
Cock	949	left T 20- 9-43: + ; right F 3-11-43	—	„ „	+ (swelling of follicles and skin)
Chicken	986	left T 27-10-43: + ; right T 3-11-43	—	„ „	+ „
Chicken	996	right T 3-11-43	+	„ „	+ (swelling of foll., oedema of the skin)
Cock	969	left T 5-10-43: + ; right C 3-11-43	+	„ „	+ „
Pigeon	909	left T 5-10-43: + ; right T 3-11-43	—	right „	+ (swelling of follicles and skin)
„	893	left T 5-10-43: + ; right P 3-11-43	+	„ „	+ (swelling of the follicels)
„	894	left T 5-10-43: + ; right C 3-11-43	+	„ „	+ „
Canary	728	left T 5-10-43: — ; right C 3-11-43	+	left „	+ many pox
„	764	C intramuscularly 0,05 cc tissue culture 1-10-43		left „	+ two pox
Canary	725	control		left „	+ many pox
Chicken	466	control		left and right thigh	+ many pox

T = turkey-pox virus
F = Fowl-pox virus
P = Pigeon-pox virus

*) The birds numbered 901 to 910 inclusive were reinfected intramuscularly with cow-pox on 9-10-

ith turkey-, pigeon-, canary-, and fowl-pox virus, proved to be sceptible to R-P. In another experiment, hen 350 was immunized trafollicularly on 20-7-43 with R-P from a 14th guinea-pig assage. A distinct local reaction developed. On 26-8-43, this bird as reinfected with pigeon-pox and again reacted.

These experiments with mammals (guinea-pigs, rabbits, calves) nd birds (hens, pigeons, canaries) prove that R-P is not a fowl-pox rus but is immunologically related to cow-pox. The skin lesions aused by R-P in birds are similar to the lesions of fowl-pox and R-P lesion on the foot-sole of a guinea-pig is similar to a cow-ox lesion in this animal (The differences have already been ointed out).

In calves the slight R-P reaction has some resemblance to a ow-pox lesion. We were as yet unable to obtain typical pock-uptions in the rabbit. No pock-like lesion was observed by either ne attending veterinarian or myself in the case of the spontaneous -P with high mortality. About 100 rabbits died from a septicaemia the course of our experiments. A typical pock-eruption was ever observed. However, when the experiments had brought to ght the relationship with cow-pox, a careful search was made or possible small, pock-like lesions, and indeed it was noticed hat some of the rabbits had a few very small, round spots at the ansition from skin to mucous membrane of the nose and mouth, hich might be considered as pock-lesions. Spots like these are metimes also found on the tongue. Necrotic spots of this kind ave been mentioned before (6). The most important post-mortem ndings have already been mentioned a few times (subcutis oedema, ght yellowish spots in the liver etc.). A separate report on the athologic-anatomical and histological changes will be published ter. Although the experiments described above have given us a earer insight into the question of the R-P virus, there are still any problems yet unsolved.

For instance: is it possible to cause typical pock-lesions in the bbit by some other method? The reverse is possible as cow-pox an cause a septicaemia in rabbits under certain conditions. Ac-ording to TOPLEY and WILSON (20) very virulent strains of cow-ox cause „pocks on the lips and tongue and lesions in the lungs, ver and other organs". However they also mention „a widespread uption". The war made it impossible to make more experiments; uring the last few years it was even difficult to obtain literature.

In connection with the results we obtained, the following facts, iven by other authors, may be of interest.

KASAHARA and his collaborators noticed changes of the testicles rabbits, while making experiments with rickettsiae. These hanges, oedema and haemorrhagic necrosis of the testicles, were aused by a filterable virus that was demonstrable in the spleen nd in the blood. In the rabbit eye this virus causes the same sions as the small-pox virus.

Monkeys and guinea-pigs are susceptible. It causes a meningo-

encephalitis when injected into the brain of a mouse. Immunologically this virus is „dem Pockenvaccinevirus sehr nahestehend". The authors are of the opinion that this virus disease is spread in rabbits „als Epidemie in einer Art unscheinbaren Infektion". KASAHARA's virus was mentioned in an earlier paper (5). The original publication was not obtainable, the short report (7) we read gave but an incomplete survey of the virus. However the information given about this virus led to the conclusion that it is somewhat similar to the R-P virus.

In 1940, FUST (8) described an outbreak of rabbit-pox. Ten days after the purchase of 27 rabbits, one of the animals had „seröseitriges Schnupfen". Twelve days later, 6 rabbits had „Rhinitis serosa oder serosa-purulenta", and some others also a „hochgradiges Schnauzenödem". Closer inspection showed „Mehr oder minder zahlreiche Hirsekorn bis linsengrosze papustulöse zum Teil zentral gedellte Effloreszenzen" on the lips, the cleft in the upper lip, sometimes on the tongue and the mucous membrane of the mouth. After 4 weeks 16 animals were „Mehr oder weniger schwer von der Seuche befallen". Four animals with very serious eruptions on the mucous membranes, developed papulo-pustular, crusty lesions of the skin, especially of the back, breast, ears and external genitals, that healed with shedding of the hair and scar forming. Two of these animals also had a „ziemlich heftige conjunctivitis". All the rabbits were seriously ill. Three died when the lesions of the mucous membranes and the skin were almost healed. Postmortem, swollen lymphatic glands were found, and „gedellte Stipchen" in the testicles, spleen, adrenals and lungs. This disease „besitzt so wohl in ihren klinischen Verlauf, als auch in ihren pathologisch-anatomischen Manifestationen gewisse Ähnlichkeit mit den menschlichen Pocken bzw. mit Vaccine, unsere Beobachtungen stehen jedoch keineswegs vereinzelt da; LEVADITI, NICOLAU und KOPCIOWSKA, BARDACH, BLANC und CAMINOPETROS, DURANREYNALS GREENE, PEARCE, ROSAHN und HU haben in den letzten Jahren analoge Kaninchenseuchen beschrieben und wegen ihren klinischen, anatomischen und immunologischen Verwandschaftsbeziehungen zu den menschlichen Pocken mit dem Namen Kaninchenpocken belegt". FUST experimented with sterile blood collected by heart puncture of „einen spontan von zahlreichen Haut- und Schleimhauteffloreszenzen befallenen Tiere am 3 Krankheitstage". With this blood a rabbit was infected; 1 cc in each testicle: the rabbit remained normal and was castrated after 4 days. The testicles were ground fine and another rabbit was infected in both testicles with this material. This animal reacted the following day and died on the 4th day. Continued passages always resulted in an haemorrhagic orchitis. The virus was compared with the rabbit-pox virus of TEN BROECK (Rockefeller Institute for Medical Research in Princeton). Two young rabbits that had sustained the disease, were not susceptible to a corneal infection with rabbit-pox virus. Two other rabbits, with the same history, were immune to

corneal infection with cow-pox. FUST also reports that 4 young rabbits inoculated with cow-pox on the cornea or in the testicles, were not immune to a corneal rabbit-pox virus infection. ,,Diese Versuchsergebnisse bestätigen die Beobachtungen anderer Autoren'' (Reference is made to the J. Exp. Med. Am. **63**, 352, 1936). The author continues: ,,Kaninchenpocken Reconvaleszenten sind immun gegen eine Nachimpfung mit Vaccinevirus. Mit Vaccine vorbehandelten Tiere sind nicht immun gegen eine nachfolgende Einverleibung von Kaninchenpockenvirus. Daraus geht hervor, dasz zwischen Vaccinevirus und Kaninchenpocken enge verwandschaftliche Beziehungen bestehen. Kaninchenpocken und Vaccine sind jedoch nicht identisch''. Rabbit-plague has many important points in common with the disease described by FUST.

Important differences are the much greater mortality in the case of rabbit-plague, and the distinct pock eruptions in the case of rabbit-pox, while no lesions of the liver occur in the latter disease. Immunologically the relationship between rabbit-pox and cow-pox seems to be exactly the reverse of that between R-P virus and cow-pox. In his book on rabbit diseases, SEIFRIED (9) describes rabbit-pox in great detail and mentions not only the first French workers on this subject (LEVADITI and his colloborators), but also the more recent American authors. Especially the latter have made extensive investigations. The following results are the most important. In 1934 GREENE (10) described an outbreak of a disease in rabbits of the laboratories of The Rockefeller Institute for Medical Research. The origin of the infection could not be traced. Experiments had been made with neuro-vaccin. The symptoms were: lymphadenitis, ,,lesions of the skin and mucous membranes'', *viz.*, variegation followed by papillae, especially on the ears, lips and eyelids, moreover many oedemas. In the eyes ,,diffuse keratitis with corneal ulceration was common'', iritis, iridocylitis and purulent ophthalmia also occured. ,,A nodular or diffuse orchitis with oedema of the scrotum'' was observed. After recovery the fertility was almost always normal. The incubation period was from $4\frac{1}{2}$ to 9 days after contact. ,,The majority of the affected animals showed generalized lesions'', *viz.*, eruptions of the skin and the mucous membranes. Sometimes only swollen lymphatic glands, swollen testicles or ophthalmia was observed. Many very seriously diseased animals recovered, although they retained scars where the corneas or ears had been inflamed. Later GREENE (11) reported on the post-mortem findings. These partially correspond to the findings in the case of R-P. For example, GREENE mentions the same milliary lesions in the liver. An important difference, however, is the pock-like form of the disease. This was never seen in spontaneous cases of R-P, and almost never in experimental cases. Furthermore GREENE mentions that the feces are hard. In R-P on the contrary, the feces are always too soft and slimy, nor does GREENE mention the haemorrhagic adrenal glands. The causal agent of the disease described by GREENE (12) is a filterable virus, isolated by PEARCE,

Rosahn and Hu. This virus is one of the pox group, and is ,,closely related to vaccinia virus but not identical with either the neurotropic or dermotropic form". Greene says that the outbreak of this disease was ,,an epidemic much like small pox in man". In his most recent publication Greene (13) gives some facts about the susceptibility of different breeds to the virus. Rosahn and Hu (14) describe the spontaneous disease occuring in 1933—34 as ,,a generalized papular eruption". Among local clinical signs the most striking were skin and mucous membrane lesions, which were noted in all cases. Pearce, Rosahn and Hu (15) report that almost the entire colony of 1400 rabbits was ill of this disease that was so much like ,,small pox of man . . . . that it was called rabbit-pox". Many serious cases recovered. The mortality was highest among the younger animals and lowest (15 %) among the adult animals. The authors are of the opinion that little has been published about this disease. They mention only a book for breeders by Mahlich, in which rabbit-pox is described as a disease that seldom occurs, and that is characterized by ,,a generalized cutaneous eruption". Pearce, Rosahn and Hu write that the rabbit-pox virus is related to cow-pox but, ,,it was not completely identical with the two specimens of dermovaccin and one of neurovaccin used for comparison". Furthermore, the prophylactic inoculation with dermovaccin offered protection against the spontaneous disease. This corresponds very well with our results, and not with the results published by Fust. Pearce, Rosahn and Hu infected rabbits in the testicles causing orchitis, fever and a quick death (3 to 5 days after the infection). Other methods of infection caused a disease with a slow course and the symptoms of a spontaneous pox infection. The same authors (16) say of the cornea infection: ,,Attempts to produce lesions of the cornea in lines of scarification were unsuccessful"; some clouding of the cornea was observed and Guarnieribodies could be demonstrated. They then investigated the relationship with vaccinia (17). It had already been found that animals that had sustained the spontaneous or the experimental infection, were immune to reinfection. Five rabbits that had recovered from rabbit-pox, were infected with virulent cow-pox. Four animals remained normal, the fifth showed an uncertain reaction. Conversely, rabbits that had sustained cow-pox were infected with rabbit-pox. The authors' opinion of these experiments follows: ,,The results of these two groups of experiments show first, the highly refractory state of recovered rabbit-pox animals to culture vaccin virus injected intratesticularly, intradermally or intravenously. These findings point to some relationship between pox virus and vaccine (culture) virus". Furthermore it was found that the rabbit-pox virus gave more distinct lesions in the calf than cow-pox gave. These authors (18) also demonstrated that cow-pox serum does not entirely neutralize rabbit-pox virus, but that rabbit-pox serum does neutralize cow-pox. They also report on experiments with mice (19) — intracerebral infection with filtered

material gives no results, unfiltered material does — and with guinea-pigs. It proved possible to cause lesions in these animals. Three days after the infection of scarifications of the hide, calves showed many small, pink papillae, which became larger and some of which had a haemorrhagic center. The authors wonder if this is a separate „pox disease of rabbits" and are inclined to think this is true. They say that no reports have been published on real spontaneous cases of rabbit-pox; „a search of the medical and veterinary literature has failed to reveal any reference to rabbit-pox as such. On the continent of Europe, however, a disease of the rabbit is recognized which is called Pocken and which greatly resembles or is identical with pox as it appeared in our colony". They refer to P. MAHLICH, Unsere Kaninchen, Berlin 1919 (FUST's report appeared after the American investigations). From the facts just mentioned, it is apparent that the R-P virus, the rabbit-pox virus, FUST's Kaninchen-pocken virus and KASAHARA's virus are all related to cow-pox, but are probably not identical with it. Whether these viruses are themselves identical, might only be ascertained by comparative experiments under identical conditions.

At present all we can conclude is that the R-P virus seems to be the most virulent. It causes septicaemia and no or almost no pox. Perhaps there is a difference in antigenic structure between these viruses. It is worth mentioning that no experiments with cow-pox had been made in the laboratory where the spontaneous outbreak occurred. No one of the staff that had been in contact with the rabbits had been recently vaccinated, nor was this the case with any member of their families.

Summary.

A spontaneous acute disease with high mortality was observed in rabbits. It was proved to be caused by a filterable virus. The virus was demonstrated in blood, urine, bile and mucus of the nose. It was possible to transmit the disease to rabbits by subcutaneous-, intracutaneous-, intranasal-, conjunctival-, intravenous infection and infection per os. The results of experiments with contact infection were quite often positive.

The virus could be cultivated on the chorio-allantois of chicken-embryos (haemorrhagic inflammation of the chorio-allantois). Brain-passages in mice were successful (mortality almost 100 %); mice that were injected subcutaneously had no symptoms clinically, but later they proved to be immune to intracerebral infection.

Guinea-pigs may be easily infected through scarifications on the foot-sole, the local pock-like reaction is not followed by a generalized eruption, even after further guinea-pig passages. The feather-follicles of fowls, pigeons and canaries proved to be susceptible to the virus; they show a pock-like reaction.

Cow-pox immunizes guinea-pigs completely against the virus of rabbit-plague; the immunity to cow-pox after rabbit-plague is

evident, but is not always complete. Cow-pox immunizes rabbits effectively against rabbit-plague; the reverse could not be demonstrated in our experiments. In cattle the relationship in immunity between rabbit-plague and vaccine was proved. There seemed to be no relationship with fowl-pox, pigeon-pox, canary-pox or turkey-pox.

The spontaneous as well as the experimental cases — more than one hundred — in rabbits were of a septicaemic and haemorrhagic character, typical pock eruptions were never noticed to any extent, not even after cutaneous or intracutaneous infection; in some cases only a few small eruptions, somewhat like pox, were seen on the lips and tongue.

The spontaneous outbreak occurred in the rabbit-stock of a laboratory; experiments with pox virus had never been made there; the staff and their families had not been vaccinated during the last few years.

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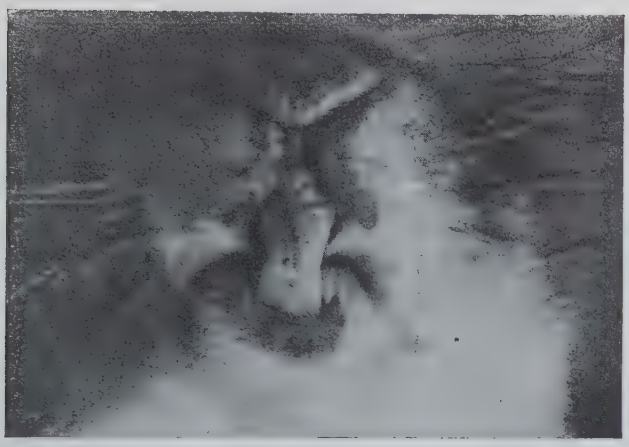

Fig. 1. Rabbit. Experimental infection. A few pock-like nodules on the lips.

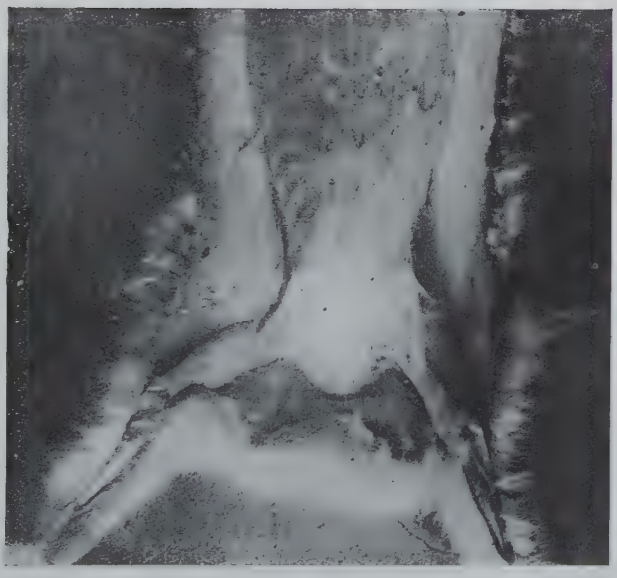

Fig. 2. Rabbit. Experimentally infected by subcutaneous infection in right thigh. An extensive moist oedema has developed at the site of injection. A large amount of liquid has flowed on to the dissection-table.

Fig. 3. Rabbit liver. Experimental infection. The organ is disseminated with small foci.

Fig. 4. Foot of a guinea-pig. Infection on the foot-sole. A swollen vesicle has developed.

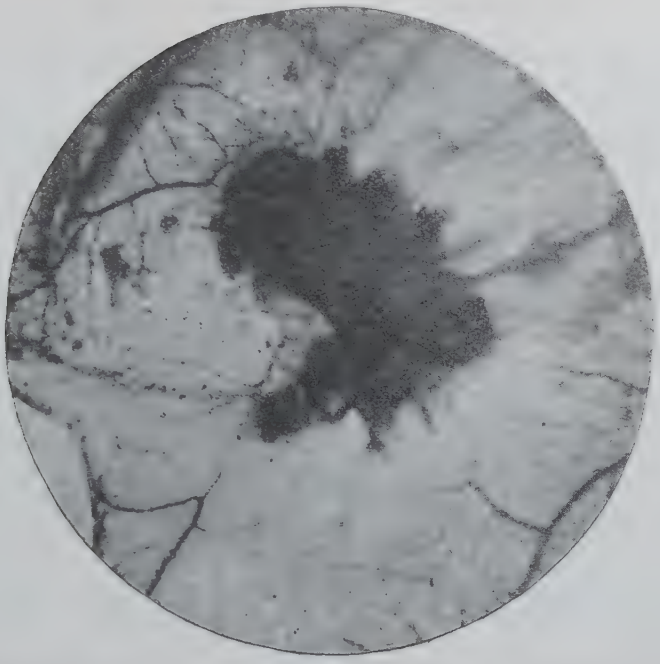

Fig. 5. Chorioallantoic membrane of a chicken-egg. Haemorrhagic inflammation. 45 Times enlarged.

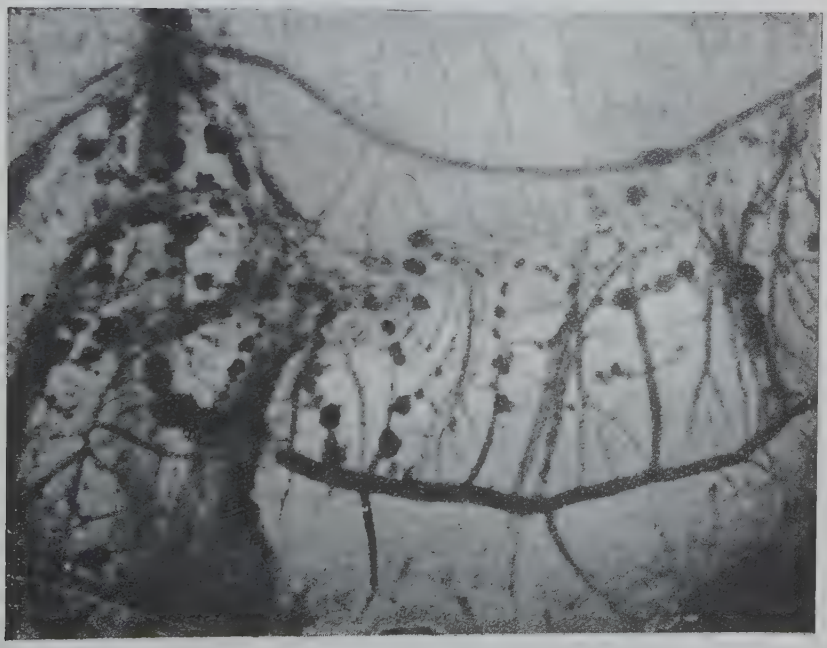

Fig. 6. Chorioallantoic membrane of a chicken-egg. Many haemorrhages along the blood-vessels. 45 Times enlarged.

(From the City Health Department, Amsterdam).

WEIL'S DISEASE IN AMSTERDAM DURING THE WAR

by

A. CHARLOTTE RUYS

(Received February 26, 1946).

Before the war the frequency of WEIL's disease in Amsterdam had its peak in the late summer and early autumn, but it did prevail throughout the whole year. Most of the cases in summer were due to bathing in contaminated water. The patients in the colder season were to a large extent infected by a fall into the rat infestated Amsterdam canals. Occupational infections were seen throughout the whole year.

From 1940 on the frequency of WEIL's disease changed as a consequence of war conditions. Table I gives the figures from 1935 till 1945.

Table I.

Cases of WEIL's disease in Amsterdam.

year	1st quarter	2nd quarter	3d quarter	4th quarter	total	water accidents
1935	0	0	4	0	4	1
1936	0	0	4	2	6	1
1937	0	6	10	0	16	4
1938	1	2	7	1	11	2
1939	0	0	7	3	10	?
	1	8	32	6	47	
1940	1	2	7	6	16	9
1941	4	2	6	10	22	17
1942	4	0	5	7	16	10
1943	3	0	3	5	11	7
1944	2	0	0	6	8	6
1945	0	1	7	2	10	2
	14	5	28	36	83	51

The black-out caused many water accidents and so cases of WEIL's disease accumulated in the last quarter of the year. In the

second quarter the fewest cases were noticed. People had to be at home at 11 o'clock or earlier, so in these months the black-out did not give so much danger for water accidents. When the curfew began at 7 o'clock, i.e. in the winter and spring of 1945 no case of WEIL's disease was noticed.

A curious thing was the total absence of WEIL's disease in the summer 1944. Had there been fewer swimmers than in other years? WEIL's disease can seldom be traced to the official Amsterdam swimming pools, but it is often caught in the river and canals in the neighbourhood of the town. In the summer of 1944 many men stayed at home for fear of razzia's by the German police and many women were overcrowded with work because of the difficulties in obtaining the necessary food. But in the holiday season the weather had been magnificent and the figures from the open swimming pools indicated that there had been even more visitors than the year before. Complaints about rat infestation were more numerous than in 1943.

There was, however, another difference as compared with 1943. For fear of the arrival of the allied troops the Germans prepared the inundation of the neighbouring polders and filled the Amsterdam canals with highly brackish water, which penetrated widely into the surroundings of the town.

The salinity of the water of the river Amstel *) at some distance of the town was in 1944 as follows:

Tabel II.

	mg Cl/litre		mg Cl/litre
January	481	July	1098
February	532	August	1572
March	741	September	1642
April	846	October	1329
May	720	November	481
June	751	December	303

In other years the salinity of the water noticed in the month of August at the same place was:

Table III.

	mg Cl/litre
1942	805
1943	639
1945	1045

*) I thank Dr. N. L. WIBAUT-ISEBREE MOENS for providing me with these data.

In previous experiments (cf. W. SCHÜFFNER, Trans. Roy. Soc. Trop. Med. and Hyg. **28**, 1, 1934) I had observed that the survival of leptospira's in water is influenced by its salinity. Even in North Sea water with 14.000 to 17.000 mg Cl/litre leptospira's did survive for some hours, but roughly taken with decreasing salt content the survival time increased. The salt does not kill the leptospira's but living conditions are less favourable.

I think that the absence of WEIL's disease during the summer of 1944 can be explained by the brackishness of the water in the town as well as in the neighbourhood, which exceeded that of other years considerably.

Summary.

During the war the incidence of WEIL's disease in Amsterdam had its peak in the winter months as a consequence of the black-out which caused many water accidents. In the summer of 1944 no case of WEIL's disease was noticed probably owing to the high salinity of the water in the town and in the neighbourhood.

(Laboratoires de chimie organique et de microbiologie de l'Université de Groningue).

DÉRIVÉS SIMPLES DE L'ACIDE p-AMINOBENZOÏQUE AGISSANT COMME SULFONAMIDES ET ANTISULFONAMIDES

par

J. L. SIRKS

(Reçu le 18 décembre 1945).

L'hypothèse concernant l'action inhibitrice des sulfonamides sur les bactéries, la plus en vogue, est celle énoncée par WOODS (32) et FILDES (9) et développée par KUHN (18) et ses collaborateurs.

Selon cette hypothèse l'acide p-aminobenzoïque (vitamine H') joue un rôle indispensable dans le métabolisme des bactéries, soit comme co-ferment, soit comme substrat d'une réaction enzymatique. Il se lie réversiblement à un porteur protéique, l'apoferment. Grâce à l'analogie de leur structure chimique, la sulfanilamide est à même de remplacer l'acide p-aminobenzoïque dans la combinaison protéique, sans cependant pouvoir remplir la fonction physiologique, de sorte que la multiplication des bactéries cesse.

Cette théorie s'appuie sur la découverte de l'antagonisme entre les sulfonamides et l'acide p-aminobenzoïque et sur le fait que la présence de substances qui ressemblent chimiquement au substrat spécifique d'une réaction enzymatique, peut empêcher cette réaction [1]).

La probabilité d'exactitude de la théorie s'est augmentée par la découverte d'antagonistes contre l'action de plusieurs vitamines bactériennes (acides pantothénique (18) et nicotinique (8, 9), lactoflavine (23)).

Si la théorie est exacte, on peut s'attendre à trouver, à côté des sulfonamides, d'autres composés ressemblant à l'acide p-aminobenzoïque et contrariant son action.

En effet KUHN a décrit de telles substances, dérivant de l'acide p-aminobenzoïque par substitution dans le groupe carboxyle (p.e. 4,4'-diaminobenzophénone (20), 4,4'-diaminobenzil (19), p-aminoacétophénone (19)).

Il nous a paru intéressant d'examiner des dérivés simples de l'acide p-aminobenzoïque, ayant des substituants dans le noyau benzénique.

[1]) Voir A. LWOFF, F. NITTI et Mme J. TRÉFOUËL, Ann. de l'Inst. Pasteur **67**, 173, 1941.

Jensen et Schmith (14) attribuent une ,,action sulfonamide typique'' à des composés qui exercent ,,in vitro'' une action bactériostatique qui est supprimée par l'acide p-aminobenzoïque.

Nous nous sommes posé pour une série de dérivés de l'acide p-aminobenzoïque les problèmes suivants:

1. Le composé exerce-t-il une action bactériostatique?
2. Si oui, cette action est-elle supprimée par addition d'acide p-aminobenzoïque?
3. Si non, peut-on suspendre l'action bactériostatique de la sulfanilamide?

La méthode de recherche dont nous nous sommes servis a été élaborée dans ces laboratoires par Keverling Buisman (17), par analogie avec un procédé décrit par Jensen et Schmith.

1. Partie expérimentale.

Méthode.

La bactérie dont nous nous sommes servis était le *Diplococcus pneumoniae* type I souche ,,Amerika'' (25); elle a été misse à notre disposition par le docteur A. E. Beute, directeur de l'institut d'hygiène.

On la cultive dans un bouillon sans peptone et additionné de 6 % de liquide ascites. Les composés qui se sont montrés actifs, seront soumis plus tard à des expériences quantitatives dans un milieu de culture synthétique et en présence d'autres micro-organismes.

Nous avons préparé des solutions étalon des sels sodiques des acides dans de l'eau stérile, renfermant, par 10 cm³, 60 mg de l'acide et une quantité équivalente de soude caustique.

5 cm³ de bouillon ascites sont additionnés de ces solutions étalon et dilués avec de l'eau stérile à 6 cm³; la concentration de l'acide varie de $^1/_{1250}$ à $^1/_{5000}$ g par cm³. La solution est ensemencée au moyen d'une dilution ($1 : 10^6$) d'une culture de pneumocoques dans du bouillon, agée de 8 heures. En même temps on fond deux plaques à compter avec 0.2 cm³ des dilutions $1 : 10^6$ et $1 : 10^7$ respectivement; les plaques se composent de 10 cm³ d'agar bouillon, additionnés de 1 cm³ de liquide ascites et d'une solution de 3 mg de p-aminobenzoate de sodium dans 0.5 cm³ d'eau. En comptant les colonies après 2 × 24 heures, on constate que l'ensemencement a été fait avec 500 bactéries en moyenne.

Les tubes de cultures sont placés dans une étuve à 37°C. et leur état trouble est examiné à l'œil nu d'abord après dix heures et ensuite toutes les deux heures.

Si le composé exerce une action bactériostatique, on répète l'expérience en présence d'acide p-aminobenzoïque. Pour les composés non-bactériostatiques on examine s'ils empêchent l'action de la sulfanilamide, en les ajoutant avec ce chimiothérapeutique au milieu de culture.

Chaque série d'expériences se compose de 10 à 15 tubes de culture; un tube ne renferme que 5 cm³ de bouillon ascites, 0.2 cm³

de culture de pneumocoques et 0.8 cm³ d'eau, un autre tube est additionné de sulfanilamide (1 : 5000).

Préparation des acides.

Nous allons décrire la méthode de préparation des acides, pour montrer comment nous nous sommes assurés de leur pureté.

1. Acide 3-hydroxy-4-aminobenzoïque.

On le prépare selon BEYER (2) en nitrant l'acide 3-hydroxybenzoïque et en réduisant le produit à l'aide de sulfure sodique en solution alcaline. On le purifie en le dissolvant dans une solution de carbonate sodique et en le reprécipitant. L'acide fond, après purification par l'intermédiaire du sel sodique, à 200°C.

2. Acide 2,4-diaminobenzoïque.

On peut oxyder le dérivé diacétylé du 2,4-diaminotoluène au moyen de permanganate potassique en présence de sulfate de magnésium (34). L'acide libre étant assez instable, on le prépare ex tempore en hydrolysant le composé diacétylé au moyen d'acide chlorhydrique alcoolique (29). Point de fusion avec décomposition: 138—140°C.

3. Acide 3,4-diaminobenzoïque.

Le 3-nitro-4-acétylaminotoluène est oxydé au moyen d'une solution de permanganate de potassium en présence de sulfate de magnésium (33) et après élimination du groupe acétyle, l'acide 3-nitro-4-aminobenzoïque est réduit au moyen de poudre d'aluminium et d'une lessive diluée de soude caustique (29).

On purifie le produit en le dissolvant dans de l'acide chlorhydrique dilué et en le faisant reprécipiter au moyen d'acétate de sodium. Point de fusion: 210—211°C., avec décomposition.

4. Acide 2-chloro-4-aminobenzoïque.

L'oxydation du 2-chloro-4-nitrotoluène donne l'acide 2-chloro-4-nitro-benzoïque (24), que l'on réduit à l'aide de sulfate de fer et d'ammoniaque (28). En faisant recristalliser le produit dans de l'eau, on obtient des aiguilles blanches, fondant à 216°C.

5. Acide 2-bromo-4-aminobenzoïque.

Le dérivé diazo du 2-amino-4-nitrotoluène est décomposé au moyen de bromure cuivreux et d'acide bromhydrique; puis le 2-bromo-4-nitrotoluène est réduit et après acétylation du groupe amino le groupe méthyle est oxydé au moyen de permanganate potassique (3). Purifié par recristallisation dans de l'eau, le produit fond à 200—201°C.

6. Acide 2-nitro-4-aminobenzoïque.

On le prépare en oxydant le 2-nitro-4-acétylaminotoluène par du permanganate potassique en présence de sulfate de magnésium

et en éliminant le groupe acétyle au moyen d'une lessive de potasse caustique (4). Recristallisé dans de l'acide acétique dilué, le produit fond à 229—231°C. avec décomposition.

7. Acide 3-nitro-4-aminobenzoïque.

Le 3-nitro-4-acétylaminotoluène est oxydé à l'aide de permanganate en présence de sulfate de magnésium; ensuite le groupe acétyle est éliminé par hydrolyse en solution acide (29). On purifie le produit en le dissolvant dans une lessive diluée de soude caustique et en le faisant reprécipiter par addition d'acide chlorhydrique; il fond à 284°C.

8. Acide 3-méthyl-4-aminobenzoïque.

Le m-xylène est nitré par un mélange d'acide nitrique et d'acide nitrosylsulfurique (31); l'un des deux groupes méthyle est oxydé au moyen de bichromate potassique et d'acide acétique (11) et ensuite le groupe nitro est réduit au moyen d'étain et d'acide chlorhydrique (1). Recristallisé dans l'eau, le produit fond à 168°C.

9. Acide 3,5-diméthyl-4-aminobenzoïque.

Le mésitylène est nitré, l'un des groupes méthyle est oxydé au moyen d'acide chromique et le groupe nitro est réduit à l'aide de sulfate ferreux et d'ammoniaque (33). Après recristallisation dans l'alcool, le produit fond à 243—244°C.

2. Examen bactériologique.

Nous avons constaté qu'une ,,action sulfonamide typique'' est manifestée par les acides 2,4- et 3,4-diaminobenzoïques.

L'action bactériostatique des sulfanilamides est suspendue par les acides 2-chloro- et 2-bromo-4-aminobenzoïque.

Les acides suivants n'ont pas d'action bactériostatique ou leur action est extrêmement faible; ils n'empêchent pas non plus l'action bactériostatique de la sulfanilamide: acides 3-hydroxy-, 2-nitro-, 3-nitro-, 3-méthyl- et 3,5-diméthyl-4-aminobenzoïque.

Lorsque les recherches que nous décrivons ici étaient terminées, nous avons pris connaissance des mémoires de Wijss, Rubin et Standskov (35) et de Johnson, Green et Pauli (15) sur l'action de dérivés de l'acide p-aminobenzoïque sur des bactéries. Par suite de la guerre, l'abstrait signalé ne nous est pas parvenu plus tôt.

Le tableau suivant réunit nos résultats et ceux que les auteurs cités ont obtenus pour les acides décrits ci-dessus.

Ainsi, nos résultats correspondent en général à ceux des auteurs cités. Seulement, pour l'acide 2-chloro-4-aminobenzoïque, qui, selon ces auteurs, serait bactériostatique, nos résultats sont contraires.

Wijss et ses collaborateurs trouvent une action antisulfonamide pour les acides 2-fluoro-, 2-bromo- et 2-iodo-4-aminobenzoïques; nos expériences sur l'acide 2-bromo-4-amino-benzoïque confirment ce résultat. Il n'est donc pas probable que le composé chloré exerce une action bactériostatique.

Acides	Wijss	Johnson	nos résultats
3-hydroxy-4-aminobenzoïque	0		0
2,4-diamino- ,,	+		+
3,4-diamino- ,,	+		+
2-chloro-4-amino- ,,	+	+	—
2-bromo-4-amino- ,,	—		—
2-nitro-4-amino- ,,	0		0
3-nitro-4-amino- ,,	0		0
3-méthyl-4-amino- ,,	0	+	0
3,5-diméthyl-4-amino ,,			0

+ = Action bactériostatique, suspendu par l'acide p-aminobenzoïque.
— = Action anti-sulfonamide.
0 = Ni action bactériostatique, ni action anti-sulfonamide.

Résumé.

Pour plusieurs dérivés de l'acide p-aminobenzoïque, substitués dans le noyau, on a examiné l'activité sulfonamide ou anti-sulfonamide sur des pneumocoques ,,in vitro".

Les acides 2,4-diamino- et 3,4-diamino-benzoïques exercent la même action bactériostatique que la sulfanilamide. Les acides 2-chloro- et 2-bromo-4-aminobenzoïques empêchent l'action inhibitrice de la sulfanilamide. Quelques autres dérivés ne présentent nulle activité.

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(From the Neurological-Psychiatric clinic of the St. Canisius Hospital at Nijmegen).

VIRUSMENINGITIS

I. CLINICAL PART

by

J. J. G. PRICK

(Received February 16, 1946).

Among the different forms of meningitis the one which develops a large number of lymphocytes in the cerebro-spinal fluid is undoubtedly the one whose aetiology provides most difficulties.

Of a number of these cases it has still to be confessed that the aetiology cannot be shown. In the cause of the last few years, however, the percentage of cases of lymphocytic meningitis, the aetiology of which is not known has diminished, because it has been discovered, that affections of the central nervous system, like acute poliomyelitis, by no means always pursue their course in the stereotyped way, but may reveal themselves as lymphocytic meningitis.

But it seems to us that some people are apt to go too far when they identify nearly every case of lymphocytic meningitis, „e causa ignota" with an a-typical form of poliomyelitis acuta anterior. As a rule we can safely draw this conclusion only during epidemics of poliomyelitis.

While consequently a certain number may be isolated from the group of lymphocytic meningitides, as being caused by the poliomyelitis virus, there is another number which is not caused by a virus-contagion, but by a bacterial infection. The latter group of lymphocytic meningitides we do not wish to discuss here.

Of one remarkable case of lymphocytic meningitis, which in our opinion was not a case of poliomyelitis and to which we were not at first able to assign another cause, we have made an extensive experimental examination, and we have managed to define it more exactly. The clinical picture of this case is as follows.

Anamnesis: Mr. A., aged 26, undergraduate, was taken ill on Juni 11, 1944 with a headache, which was diffused through the whole head, but was localized mainly behind both eyes; the temperature was only slightly higher than normal. Gradually the headache became intenser, while the patient also began to complain of pain in his neck. Three days after the first symptoms the patient became aware of diplopia. The temperature had in meantime risen to 101° F. After another day the patient began to feel drowsy, and sick, but he did not vomit. From the first there was costiveness.

From what he told us it appeared that he had suffered from a

tuberculosis of the lungs in 1936. From 1937 he must have been in good condition again. The patient says that at present he does not cough or expectorate and does not feel tight in the chest. He does not know about having been ill previously or having been in contact with diseased people; nor had he been swimming of late. It is of importance to know, however, that shortly before his illness the patient had caught a mouse, which he held in his hand for a while. The family-anamnesis tells us that some members of the family had a sensitive nervous system.

Status on admission: The patient who had been taken ill at home five days before, shows on admission into the neurological clinic a rectal temperature of 111° F., while the pulse-rate is 118 per minute. Although the patient looks ill indeed, he has full command of his intellectual faculties. There is no icterus, no cyanosis, no dyspnoea, no oedema. His pulse is well-filled, not tense, not celer, equal, regular, left and right identical.

During the examination we were immediately aware of the existence of meningeal irritation. The patient lies in opisthotonos, has strikingly red auricles with intense painfulness on pressure below the auricles near the foramen stylomastoideum; besides there is a rigidity of the neck and a positive symptom of Kernig and Brudzinsky.

The tongue is slightly coated, the pharynx does not show any pecularities. Nor does the heart, on examination, show any abnormalities but, on percussion the top of the left lung sounds a little muffled, while on auscultation crepitating rhonchi are to be heard.

The abdomen does not show any disturbances, in this case the abdominal wall is not retracted; liver and spleen are not palpable nor can elsewhere any palpable disturbances be found. There are no abnormalities in the extremities, nor in the spinal column. There are no gland-swellings.

The neurological examination does not show besides the above-mentioned meningeal irritation and a slight horizontal nystagmus when looking right or left, any objective irregularities. Although the patient himself declares to be affected with double vision, an objective examination detects no certain disturbance of the ocular movements. On closer analysis of the dual images they appear to stand horizontal and not to be crossed, while in looking to the left in the horizontal plane of vision the distance of the dual images becomes greater. From this it may be concluded that the left M. rectus externus which is innervated by the N. abducens is paretic.

The more specified clinical examination produced the following findings: On lumbar punction the pressure of the spinal fluid is 270 mm. Pulse- and respiratory rates are normal, while the Queckenstedt test turns out positive. The spinal fluid is colourless and clear, it does not contain any coagulation. The Pandy reaction is positive as also the Nonne-reaction.

505/3 cells are found per mm³, of which 90 % are lymphocytes. When stained either by GRAM's method or by ZIEHL-NEELSEN's no micro-organisms are found. The glucose-amount of the spinal fluid is 0,65 gr. %. The reaction of LANGE produces the following values: 0.111.000.000. The luetic reactions of the blood and of the spinal fluid are negative. On further laboratory investigation it appears that the urine is normal as also the sediment.

The sediment-rate of the blood is 2 mm after 1 hour, 4 mm after 2 hours. The amount of haemoglobin is 105, the number of erythrocytes is 4.950.000, of leucocytes 9800, differential count of the leucocytes, thrombocytes show no peculiarities. The amount of ureum in the blood is 350 mg per litre (Ambard).

The blood-culture is negative as also the urine culture. The agglutination-reactions produced the following results: Ty 1/1000, PBs 1/100, PBm 1/100, Bang negative, Proteus X 19 negative, Weil negative. Finally it should be noticed that the von Pirquet-reactions, both human and bovine are positive.

In view of the previous tuberculous pulmonary affection and the present slight disturbances in the left top of the lung the thorax was X-ray examined. It appeared, that the right hilus was a little heavier than normal although this disturbance is insignificant. The top of the left lung shows a few small spots. The mediastinum posterior is not quite sufficiently clear. The sinus pleurae are normal. The ultimate conclusion is that radiographically there are practically no abnormalities in these lungs. Taking together the different clinical data we arrive at the conclusion that our patient is suffering from lymphocytic meningitis. Although on the first day of observation it could not with certainty be decided whether this was a case of so-called meningitis lymphocytaria idiopathica or of an incipient meningitis tuberculosa, there were more motives pointing in the direction of the former diagnosis (normal glucosic amount in the spinal fluid, lucid mind, absence of chorioidal tubercles, no avowed paralyses of the basal craneal nerves, absence of tubercle bacilli).

The further development proved absolutely, that we were here confronted with a so-called meningitis lymphocytaria. During the first few days after admission there was a gradual falling of the temperature and frequency of pulse, which on the seventh day after admission had both returned to normal. Simultaneously with the fall of the temperature the meningeal irritation also disappeared, while the subjective complaint of diplopia was no longer heard of. A renewed lumbar punction a fortnight after admission showed that the pressure of the spinal fluid had reached its normal level, while as the only abnormality a slight lymphocytic pleiocytosis was assessed (34/3 per mm³).

Summing up we can say that the patient described has been suffering from lymphocytic meningitis, which there are no well-founded reasons to class without comment among the meningitic forms of poliomyelitis acuta anterior nor among the bacterial forms

of meningitis lymphocytaria (Weil's disease, Koch's disease and others). No more are there any indications for us to assume the presence of a „swineherd's disease", which occasionally runs the same course as lymphocytic meningitis.

The question now presented itself whether by means of an animal experiment we might gain a deeper insight into the nature of this lymphocytic meningitis.

The findings of this experiment will be recorded and reported on by our colleague Dr. Verlinde.

(From the Department of Bacteriology and Experimental Pathology of the Institute of Preventive Medicine at Leiden).

II. VIROLOGICAL PART

by

J. D. VERLINDE

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A so-called aseptic lymphocytic meningitis may occur in the course of various infectious diseases. Besides, we know the lymphocytic choriomeningitis, caused by the virus of Armstrong and Lillie (1). Until now this disease has not been diagnosed in Holland. During the initial fever period the virus is found in the blood. Afterwards a meningitic stage occurs which usually lasts for some days only; then the virus can be demonstrated in the cerebrospinal fluid.

The virus of lymphocytic choriomeningitis was discovered in America in 1934 by Armstrong and Lillie (1). During an epidemic of St. Louis encephalitis they tried to demonstrate the causing virus in the brain of a lethal case, by inoculating monkeys. The result was the recovery of an unknown virus that could be passed into monkeys. The virus did not cause encephalitis but choriomeningitis in the monkeys. The experiments did not prove that the virus was of human origin; it could be as well a virus occurring spontaneously in monkeys. In 1936 Rivers and Scott (7) demonstrated it with certainty in the cerebrospinal fluid of patients, suffering from a mild, lymphocytic meningitis. Traub (8) detected the virus in laboratory-mice and proved that a large number of these animals may be infected. Presumably the mice are infected very young. Generally the spontaneous infection does not cause any morbid symptoms, but after intracerebral inoculation meningoencephalitis occurs. Soon it became evident, that the virus was also distributed among wild mice and that these animals constitute the proper source of the virus. Armstrong, Wallace and

Ross (2) examined 300 mice captured in or near houses, where cases of human choriomeningitis occurred. 21 percent of these mice proved to be infected. The virus is excreted with the urine and the nasal mucus. Human beings are infected presumably by direct contact with mice or by foodstuffs, soiled by urine of these animals. It may also be possible that insects transport the virus. This succeeded experimentally with Aëdes aegypti (Coggeshall (3)). In monkeys and dogs too the virus seems to occur spontaneously.

The virus has been demonstrated not only in America, but also in England (Findlay, Alcock and Stern (4)) and in France (Lépine and Sautter (6)).

Lépine, Mollaret and Kreis (5) inoculated human volunteers experimentally. Sometimes the virus could be demonstrated in the urine.

Experimental.

Animal inoculations.

In 1943 and 1944 Prof. Prick at Nijmegen now and then sent us cerebrospinal fluid from patients with affections of the nervous system, of unknown origin. The bacteriological examinations were negative. On account of the shortage of laboratory animals only a few rabbits, guinea-pigs, rats and mice could be inoculated. These inoculations, though made by different ways, were negative.

On the first of July 1944, however, I received cerebrospinal fluid of an adult man, suffering from meningitis. The fluid was clear, and bacteriologically sterile. A culture test and guinea-pig test, in order to demonstrate tubercle bacteria were also negative.

Meanwhile a number of animals was inoculated with the fluid on the day of delivery. *a.* 2 rabbits, intracerebrally with 0.2 cc each. *b.* 1 rabbit corneally. *c.* 3 mice, intracerebrally with 0.02 cc each.

The rabbits did not react to the inoculation. From the 3 mice, 2 died however after 7 and 9 days respectively. In the brain no bacteria could be demonstrated. An emulsion of this brain was inoculated intracerebrally in 3 other mice, which all died after 10 days. In this way a series of mouse-passages could be made and the animals always died after 8—10 days. A few mice lived a little longer than 10 days, but 14 days at the utmost.

The symptoms usually started on the 6th or 7th day, sometimes 1 or 2 days later. The animals became drowsy, sat huddled up, they had bristling hair and conjunctivitis often occurred. With difficulty they could stand up, especially after lying on the flank; then sometimes they turned themselves once round the longitudinal axis, and when at last in a sitting position, the hind-legs were drawn but slowly under the body. Now and then they continued sitting for some time with their hind-legs stretched backwards. The toes were either spread out, or bent spasmodically. By lifting them up on the tail, usually a spasm of the legs, especially of the toes, occurred.

The same condition could be roused by inoculation of lungs, liver, spleen and kidneys.

The intracerebral inoculation was always lethal. After intraperitoneal or subcutaneous inoculation no or slight symptoms occurred. The animals might be drowsy and show some conjunctivitis, but cerebral symptoms were not observed. As a rule these animals survived. Nevertheless the virus could be demonstrated in the brain and in the other organs.

By inoculating Seitz EK filtrates of organ emulsions I succeeded in rousing a condition, which agreed with that mentioned above. The incubation period however was 10—17 days and the mice died 2—3 weeks after the inoculation.

The post-mortem examination did not show any macroscopically visible lesions. At the histological examination, in the liver, sometimes in the lungs and in the kidneys little foci with mononuclear infiltration were found. In the brain lymphocytic meningitis, partly also encephalitis was found. Especially the chorioid plexus and the membranes between the cerebrum and the cerebellum were infiltrated with lymphocytes, while the surface membranes showed no or little alterations. In the brain itself, especially in the vicinity of the ventricles and starting from the infiltrated pia, accumulations of lymphocytes and morbid growths of glial cells were found. Sometimes a small perivascular infiltration was found.

It may be concluded, that the experimental disease in mice is caused by an infective agent, presumably belonging to the filterable viruses. The question is whether this infective agent was present in the cerebrospinal fluid of the patient, or occurs spontaneously in mice and is activated by the intracerebral inoculation.

In the first place may be replied that such an infective agent has never been demonstrated in Holland. If it occurred in laboratory mice, it would certainly have been observed by the many intracerebral inoculations, which are made in mice in many laboratories. Yet, in every mouse passage I made a control test; 2 or more mice from the same stock were inoculated intracerebrally with sterile broth, saline or Seitz filtrates of bacterial cultures. In these control mice morbid symptoms or death never occurred. If the control mice were inoculated later with virulent material, then the characteristic disease developed.

Besides mice 3 guinea-pigs and a young dog were inoculated with virulent mouse brain. The guinea-pigs were inoculated intracerebrally, intraperitoneally and subcutaneously respectively. After 12 days they showed rhinitis and conjunctivitis, while the intracerebrally inoculated guinea-pig showed fever (41,8° C.) on the 5th day, which temperature returned to normal on the next day.

The intracerebrally inoculated guinea-pig was killed 20 days after the inoculation. The chorioid plexus was slightly infiltrated with lymphocytes. Other alterations were not found. The other guinea-pigs were killed 16 days after the inoculation. The sub-

cutaneously inoculated animal showed a beginning pneumonia, the post-mortem examination of the other was negative.

A young dog was inoculated intracerebrally with virulent mouse-brain. 11 days afterwards a slight rise of temperature occurred (39,3° C.) and by inoculating mice the virus could be detected in the blood. On the 14th day another slight rise of temperature appeared (39° C.). Other symptoms were not observed.

Cultivation.

The cerebrospinal fluid received on the first of July and since kept at —16° C., was inoculated on the first of August on the chorioallantois of eggs which had been incubated for 11—12 days, by the method of BURNET. After three more days of incubation the egg membranes were checked on bacterial sterility; kept at —16° C., and part of it was inoculated in new eggs. In this way 6 egg passages could be made. Then the virus was still virulent, but more passages could not be made for lack of eggs. As appears from Table I, the virus is present not only in the chorioallantois, but also in the organs of the embryo. Considering the duration of life of the mice, it seems that the largest concentration of virus is present in the chorioallantois. The quantity of inoculated material was always the same (0.02 cc of a 1/25 dilution).

Table I.

material	number of mice		duration of life after inoculation
	inoculated	died	
chorioallantois 4th passage	6	6	7—8 days
,, 6th ,,	4	4	8—10 ,,
embryo brain 6th ,,	2	2	11—14 ,,
embryo liver 6th ,,	2	2	10—18 ,,

Titration of virulence.

The virulence of the brain of the 4th mouse-passage and of

Table II.

dilution	duration of life in days of the inoculated mice		
	4th mouse passage	4th egg passage	6th egg passage
10-1	8	8	6—7
10-2	9	8	6
10-3	10—11	8—10	7
10-4	13—19	8	6
10-5	15—17	8—10	7
10-6	17	8	8
10-7	—	13	10—15
10-8	—	—	—

the allantois of the 4th and 6th egg-passage was titrated by inoculating 2 mice intracerebrally with 0.02 cc of each dilution of a series. The virulence appeared to be high, 10^{-6}, 10^{-7} respectively (Table II).

Immunity.

4 sera have been tested for the possible presence of specific neutralizing antibodies:

Serum B: serum of the patient, about 2 months after the onset of the disease.

Serum P: serum of a patient, suffering from polyneuritis, in whose cerebrospinal fluid no infective agent had been found.

Serum N: serum of a normal person.

Serum H: serum of the experimentally inoculated dog, about 6 weeks after inoculation.

Wanted for the neutralization test are: 1. Virus: a virulent suspension of egg membrane of the 6th egg passage was used in a 10^{-6} dilution. 2. A series of dilutions of each serum to be tested. The test was carried out as follows:

Equal parts of virus dilution and serum dilution were kept at 37° C. for 2 hours and at 4° C. for half an hour. Then 2 mice were inoculated intracerebrally with 0.02 cc of each mixture, that is a quantity of virus corresponding with 5 m.l.d.

At the same time a control test was carried out by inoculating mice with an equal quantity of a mixture, consisting of virus dilution and an equal part of saline; this mixture had also been kept at 37° C. for 2 hours and at 4° C. for half an hour. The control mice died within 14 days. All mice inoculated with virus-serum P and virus-serum N mixture, also died within 14 days, so that these sera did not contain neutralizing antibodies. In the sera of the patient and the dog however antibodies could be demonstrated.

Table III.

serum end dilution	sera			
	B	P	N	H
1 : 2	+	—	—	+
1 : 5	+	—	—	+
1 : 10	+	—	—	+
1 : 20	+	—	—	—
1 : 50	+	—	—	—
1 : 100	+	—	—	—
1 : 200	—	—	—	—

Conclusion.

From the cerebrospinal fluid of the patient an infective agent was isolated, that could be inoculated intracerebrally into mice

in several passages. In the mice a lymphocytic meningo-encephalitis developed. The infective agent was filterable through Seitz EK filters, invisible, and could only be cultured on the egg membrane. The convalescent serum and the serum of an experimentally infected dog contained neutralizing antibodies, which do not occur in normal sera. It is obvious that this is a case of infection with the virus of lymphocytic choriomeningitis of ARMSTRONG and LILLIE. Various qualities completely agree with those, already known in the virus of lymphocytic choriomeningitis:

1. The sensitiveness of mice and guinea-pigs and more or less of dogs too.
2. The incubation time and morbid symptoms in these animals
3. The histological lesions in the animals.
4. The cultivation on the egg membrane.
5. The occurrence in the cerebrospinal fluid of man, suffering from lymphocytic meningitis.

An original strain of the virus of ARMSTRONG and LILLIE not being present in Holland, the immunological identity with this virus could not be examined.

It is unknown in which way the patient had been infected. On account of his residence in Noord-Limburg, and his frequent visits to farms, an indirect infection by mice is certainly possible. As this province became field of war in the course of the experiment, the occurrence of the virus in mice could not be examined.

Summary.

From the cerebrospinal fluid of a patient, suffering from a so-called aseptical meningitis, a virus was isolated, whose characteristics completely corresponded with the virus of lymphocytic choriomeningitis of ARMSTRONG and LILLIE. The intracerebral inoculation into the mouse is always fatal, and causes a lymphocytic meningo-encephalitis. The intraperitoneal and subcutaneous inoculations are usually not fatal, and do not always contract the disease. The virus could be cultivated on the egg membrane. The convalescent serum contained neutralizing antibodies.

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THE RHESUS FACTOR

by

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LANSTEINER and WIENER reported in 1940, that an agglutinogen might occur on human red blood corpuscles which corresponds in character with an agglutinogen of a definite species of monkeys, *viz.*, the *Macacus rhesus*. They called this agglutinogen Rh, the first letters of rhesus. According to the occurrence of this factor on blood corpuscles they termed the blood as Rhesus-positive or -negative. These scientists established that 85 % of the white population in the U.S.A. was Rh-positive. In definite groups of Red Indians and Chinese this might amount to 99 %; thus in those cases the number of rhesus-negatives was very low indeed.

Soon these investigations were corroborated in other countries; tests carried out in Europe resulted in a same percentage of Rhesus-positive persons of 85. The interest in the Rhesus factor grew, as it appeared that next to its scientific value it was significant as well in clinical practice. Also in the Netherlands the attention was initially drawn to this phenomenon by its clinical aspect by VAN DER SPEK. This took place in the early years of the war and the data available from foreign literature were still very incomplete. BROMAN, however, had the kindness to mail us his publication which made it possible for us to put up experiments in this direction.

To which property is it due that the Rhesus factor has roused in such measure the interest of clinicians? This is caused by its very markedly antigenic character, which may entail pathological changes. For the sake of completeness we will mention that the elements of the blood groups A.B.M.N. thus far recognised, possess these antigenic properties as well, but in such a small degree, that they induce merely rarely complications.

In two fields this pathological action of the Rhesus factor comes to light, *viz.*, in blood transfusions and in an injury occurring in neonati, *viz.*, the erythroblastosis. In blood transfusions the injury occurs merely when blood has to be transfused repeatedly in a same recipient. According to the classical system of blood grouping donor and recipient may match exactly and still sometimes serious complications may occur. BROMAN states that among 60 of such

cases he could indicate 31 times the Rhesus factor as the causal agent. By the antigenic action of this factor transfusion of Rhesus-positive blood into a Rhesus-negative recipient will induce an agglutinin against this Rhesus factor, thus an anti-Rhesus agglutinin. When subsequently again Rhesus-positive blood is administered, a haemolysis may follow, as the Rhesus factor and its corresponding agglutinin are brought together.

After the first repetition of the transfusion, generally none but slight peculiarities may be noted. The production of the anti-rhesus-agglutinin after this first supply is mostly still too low to entail complications. From the paper of BROMAN it may be learned, however, that by close observation deviations of the normal course may be detected. The results of the transfusion may not match the expectations: either the calculated increase in haemoglobin may not be reached or no increase whatever may occur. Also some cold shiver or a rapidly passing lumbago may point at an existing incompatibility. When, however, such symptoms are not heeded, then a second transfusion will take a much more serious course, as now the amount of anti-rhesus agglutinin will have increased considerably by the second stimulus.

As for the erythroblastis foetalis, here as well the Rhesus factor plays an important part. As we are not personally acquainted with its clinical picture, we have to refer to the communication of BROMAN.

The various aspects of this disease known as icterus gravis neonatorum, anaemia neonatorum and hydrops congenita have all a same cause in common, the intensive breakdown of blood corpuscles with a resulting hyperfunction of the haemopoetic apparatus. The cause of this disease has remained enigmetic, until the detection of the Rhesus factor. The course of events is conceived as follows. A Rhesus-negative mother expects a child from a Rhesus-positive father, which thus like the father may belong to the Rhesus-positive group. Genetically this is perfectly clear. This Rhesus factor occurs according to STRATTON in a very early stage of the foetus. Taking into account the strongly antigenic action of this factor it is serologically acceptable that in the Rhesus-negative mother, as a reaction against it, an anti-Rhesus agglutinin may develop. This may occur, fortunately, however, medical practice learns that this does not occur always. 15 % of all women being Rhesus-negative and 85 % of all men Rhesus-positive, this combination of Rhesus-negative woman and Rhesus-positive man may be surely expected in at least 10 % of all marriages. Erythroblastosis, however, occurs in 1 out of 200 to 400 deliveries, so that it may be assumed with certainty, that the existence of the dreaded combination as such need not entail the harmful effect. What further conditions may act hereupon may be guessed at, but thus far nothing has been proved and we need not enter into this any further.

The anti-Rhesus agglutinin once induced in the body of the mother

will in its turn enter into the blood circulation of the child via the placenta. Thus there will occur side by side the anti-Rhesus agglutinin and the Rhesus-positive blood corpuscles. Notwithstanding this generally no haemolysis will occur in utero. It is supposed that a hormone developed in the mother acts as an inhibitor. After the partus the supply of this hormone will have come to a close and nothing will inhibit the haemolysis. In how far the anti-Rhesus agglutinin might act directly in the haemolysis (thus far in vitro no haemolysis has been induced by its means), or that another agent that would develop along with the agglutinin might be responsible for it, must be left open.

Whereupon is this assumption based? As first item has to be mentioned that experiments have shown that in over 90 % of the cases of erythroblastosis the mother was Rhesus-negative. As the number of negatives among women is 15 %, the number of more than 90 % may surely not be deemed accidental.

But more convincing is the observation that in many cases the anti-Rhesus agglutinin could be detected in the body of the mother. The fact that this is not always the case and that the strength of anti-Rhesus agglutinin does not always correspond with the degree of severity of the erythroblastosis induced does not diminish the value of this finding. Several arguments are offered to confirm it, the treating in detail of which would, however, lead us to far.

As final argument it may be stated that in races in which the number of Rhesus-negatives is very low (Red Indians and Chinese) the clinical picture of the erythroblastosis is about unknown.

It may be clear that these observations justify completely the assumption that the Rh factor plays a major part in the erythroblastosis.

Attention must still be drawn to the remarkable fact, that in those cases where the marriage will lead to erythroblastosis the clinical picture will mostly occur not earlier than in the second child and sometimes in the third child. RACE and TAYLOR state, that among 44 Rhesus-negative mothers where erythroblastosis had occurred, the first children were in 38 cases perfectly normal. It seems as if the body of the mother has first to learn to produce the anti-Rhesus agglutinin, at least in a sufficient measure, and that is mostly not attained at during the first pregnancy [1]). When a second pregnancy follows, during which the Rhesus factor of the child may again act as a stimulus, the production of agglutinin will occur in a much larger measure, with the catastrophal consequences it entails. Where a general tendency exists as to the formation of small families, not all cases of potential erythroblastosis may be recognised.

This phenomenon, however, is not of a measure to explain the stated incongruency between the number of calculated and observed cases of erythroblastosis.

[1]) The observation of a similar fact has already been mentioned for the first blood transfusion.

A question which involuntarily imposes itself is, whether any measures might be taken to prevent this erythroblastosis or to bring down its consequences to a low degree. Prophylactically merely one measure is at our use, *viz.*, never to administer a transfusion of Rhesus-positive blood to a Rhesus-negative woman who might become pregnant. This transfusion might induce the production of anti-Rhesus agglutinin in which case a subsequent pregnancy might lead directly to erythroblastosis.

Fortunately, however, therapeutically more help may be offered. First of all measures may be taken in the interest of the newborn. The cause of the disease in the child is, as we have mentioned, a haemolysis, which causes the destruction of blood corpuscles as the result of the presence of anti-Rhesus agglutinin. To substitute these lost corpuscles a blood transfusion may be administered. As a matter of fact no good is to be expected from a transfusion of Rhesus-positive blood, as this will be haemolyzed as well. It is clear that none but Rhesus-negative persons will be of value for this transfusion [1]). These transfusions have to be administered until the action of the anti-Rhesus agglutinin present in the baby has come to a close. Then the child will be able to thrive on the self-produced Rhesus-positive blood corpuscles. The possibility is to be reckoned with, that the child along with the mother milk might take in more anti-Rhesus agglutinin. It has come to light that this agglutinin may occur in the mother milk, so this risk has to be avoided in the beginning.

BROMAN puts higher demands on the donor. He gives the preference to a Rhesus-negative mother who is about 7 months pregnant and whose husband is Rhesus-negative as well. He not merely thus excludes the possibility of an anti-Rhesus agglutinin, but he puts to use moreover the hormone present in the woman, which as we have seen, would inhibit the binding between the Rhesus-positive blood corpuscles and the anti-Rhesus agglutinin.

Still in another way help may be needed, *viz.*, for the mother. When she might come in danger of fatal haemorrhage, then, seen the presence of anti-Rhesus agglutinin, neither a Rhesus-positive donor can be used. Again a Rhesus-negative donor is needed. So it is recommended, when a case of erythroblastosis is expected, to look out beforehand for a Rhesus-negative donor. Generally a donor of the blood group 0 will be preferred, as this would match mother as well as child.

A Rhesus-negative donor of the same classical blood group as the mother might do as well; whether this one would be suitable for the child would depend on the blood group of the latter. From the most recent literature, which I shall refer to later, it appears moreover that merely then the suitable donor will have actually been found, when his blood corpuscles have been tested with the

[1]) The Rhesus-negative mother as a matter of fact cannot be a suitable donor, because of the anti-Rhesus agglutinin present in the serum.

serum of the mother. In this case the test-tube method such as it has been worked out by LANDSTEINER will have to be applied. This again I shall refer to later.

For the determination of the Rhesus factor in man test serum is to be started with. For the preparation of the latter the method indicated by LANDSTEINER and WIENER has to be followed in which blood corpuscles of *Macacus rhesus* [1]) are injected peritoneally into guinea-pigs [2]). The serum of the guinea-pig obtained subsequently contains the anti-Rhesus agglutinin. The irregular agglutinins arisen along with this are eliminated by means of saturation with Rhesus-negative blood corpuscles. The production of active test serum is merely low. As a matter of fact merely few ml of serum will be obtained from each guinea-pig, and moreover by no means all of the treated guinea-pigs will produce a suitable serum. Out of 30 guinea-pigs we started with in our initial preparation of test serum merely 2 appeared to have produced a sufficiently active serum. In a second experimental set the result was somewhat more favourable, *viz.*, 2 active sera out of 10 guinea-pigs. According to DAHR human blood corpuscles with the Rh factor might be used in stead of the blood corpuscles of *Macacus rhesus*. In an experiment carried out by us which covered 30 guinea-pigs, the result was completely negative. Shortly after his cited paper DAHR communicated however that he had left this method for the preparation of serum; although in his working period at Düsseldorf it had proved successful, later during his stay at Berlin it had failed completely.

Besides the test sera mentioned there is still another source of active specific sera, *viz.*, the sera of mothers of erythroblastic children; as has been mentioned already in such serum an anti-Rhesus agglutinin can be detected. Usually these sera have the highest titre from the 5th to the 10th day after the partus.

In our experiments we made use as a rule of two guinea-pigs and two or more human sera. It appeared that the results from the sera of either source did not always agree [3]) and that both human sera neither always agreed in action. This caused much uncertainty, so that we met with great difficulties in our initial Rhesus tests. We shall see, however, that the more recent foreign literature, which reached us after the liberation, may explain this varying behaviour in human sera.

In the judging of the reactions obtained with the mentioned sera

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[1]) We could dispose of these red corpuscles thanks to the kind assistance of Dr SUNIER (Director Zoological Garden Amsterdam) and Dr KUIPER and Dr PETERS (respectively Director and Veterinary surgeon of the Zoological garden Rotterdam) to who I am much endebted.

[2]) Initially LANDSTEINER and WIENER made use of rabbits; later, however, no more use was made of them, as the anti-M agglutinin developed in these animals as well.

[3]) Thus sera of guinea-pigs gave always positive agglutination with neonati; with sera of adults the usual classification came to light again.

it has to be taken into account, that the agglutinations occurring are in most of the cases extremely slight, so slight even that merely by shaking the agglutination may break up altogether. So the slide method is not adequate and one is compelled to use the test-tube method such as it is applied by LANDSTEINER. We used tubes with a diameter of 8 mm and a length of 8 cm. 2 drops of test serum + 2 drops of a 1 % suspension of the erythrocytes to be tested are introduced into them. Experience has taught us, that this sequence — „serum-erythrocytes" — made for the best results. When sera of women are to be tested for anti-Rhesus agglutinin, then blood corpuscles are started with the occurrence or non-occurrence of the Rhesus factor on which is known. Here as well the test has not to remain limited to two samples of blood, a negative and a positive one, but it has to be extended over at least four of each kind. In need certainly not to be stressed upon, that in the judging of the reaction arrived at with the sera of woman, any eventually occurring irregular agglutinin has to be reckoned with. The putting up of controls in each determination of the test sera to be used, as well as of the test blood corpuscles, may be considered as a matter of course. When sera and blood corpuscles have been brought together, they are mixed (shaking of the tubes). Then the tubes are kept during 3 hours at room temperature and left undisturbed. The period of waiting before the reading may be taken shorter, such as it is advised in England (*viz.*, one hour at 37° C.), but thus far we have not noted any definite advantage of this method. For the time being we will apply both methods one along with the other.

When sera of women are used, one may hit once and again upon a remarkable phenomenon which is known as well in other serological reactions, *viz.*, the zone-phenomenon. This term is applied to the fact observed, that in undiluted sera no agglutination may occur, whilst it does in diluted. This is the reason why in using sera of women we always dilute them up to the half by adding one drop of salt solution to one drop of serum.

The reading of the reactions is realized in the following way. The sediment in the basal end is studied with a pocket lens (4 times magnification). It is very advantageous to apply a strong illumination, because the question of actual importance is: „What does the sediment look like?"

When in the basal end a uniform mass has been formed with an even rim and without any folds the reaction is negative, thus no agglutination has taken place. When the agglutination has occurred, however, the sediment is granular with stripes and folds, whilst the rim is coarse in aspect with offshoots and inthrusts. In typical cases the difference between agglutination and no-agglutination are very marked; intermediates between both extremes exist, however, which may cause a lot of trouble. Especially initially faults are sure to occur. In the long run one learns to master these difficulties in some measure and this is mostly fur-

thered by an increased disposal of test material. In doubtful cases the investigation may then always be extended. It gave me, however, some satisfaction to read in the War Memorandum no. 9 of the Medical Research Council, that the interpretation of these reactions is by no means simple. It is emphasised, that merely those who dispose of a wide experience may arrive at definite conclusions.

Might these Rhesus tests be carried out on a large scale? Taking into consideration the influence which is induced in blood transfusions by the Rhesus factor, this would surely be desirable, but thus far it is still impracticable. In this respect we agree with the statement in the already mentioned War Memorandum: „At present, however, this ideal (the examination of all donors) may be regarded as impracticable, because of the technical difficulties of making Rh grouping tests on a large scale". The carrying out of the Rhesus test will thus far have to be limited to:

1. those cases where a erythroblastosis is to be expected, which will entail the looking for a suitable Rhesus-negative donor.
2. those cases of blood transfusion, where this will have to be repeated several times.

Has thus the problem of the Rhesus factor been completely solved? When with the ending of the war English and American literature became available again, we soon became convinced of the contrary. The Rhesus problem is much more intricate than has been assumed initially. This did not cause us any surprise, as we as well had hit upon phenomena, which were left unexplained by the existing conceptions. We have cited already the fact that not all sera of women (mothers of erythroblastotical children) reacted in a same way. Initially we thought that this had to be ascribed to the titre of the agglutination. An investigation carried out in this aim indicated however, that a difference in quality and not in quantity existed here.

In this connection we will present a short survey of the more recent conceptions in this field in as far as we dispose of the data.

In 1942 WIENER detected the serum of a mother which did not agglutinate human erythrocytes in 85 % but merely in 70 %. In the remaining 15 % of the persons considered thus far as Rhesus-positive no reaction with this new serum occurred. Analogously with the subdivision of the classical blood group A in two undergroups A1 and A2, WIENER subdivided the Rh factor also in two undergroups Rh1 and Rh2. Rh1 agglutinated merely with the newly detected serum, whilst Rh2 covered the remaining part of the Rh group which thus did react on the anti-Rhesus agglutinin used thus far, but not on the last-detected serum. As to the genetics WIENER assumed that not, as had been claimed up till then, two factors *viz.*, Rh and rh existed, but three, *viz.*, Rh1, Rh2 and rh. The possible genotypes thus consisted out of Rh1—Rh1; Rh1—Rh2; Rh1—rh; Rh2—Rh2; Rh2—rh and rh—rh. Any dominance of one

of these genes can no longer be assumed, as, such as it will be seen presently, the occurring agglutinations depend completely on the sera started with.

Later MAC CALL, RACE and TAYLOR detected in a Rhesus-positive mother [1]) a woman's serum deviating still more strongly in its activity, termed by them St (the first letters of the name of the mother) which reacted with 80 % of all suspensions of human erythrocytes. The remarkable fact could be noted that all rh-negative persons, traced up till then (thus 15 %) and further the group of Rh2 of WIENER (15 % as well) belonged to this group, whilst the remaining 50 % was scattered over the remaining groups. In merely 20 % the result of the test with this serum was negative. RACE and TAYLOR assumed, seen the reaction with rh as well as with Rh2, that the negative results occurred in the genotype Rh1Rh1, which thus would exist in 20 % of men. Starting from this assumption it was possible for them, seen that on the one hand the type Rh1Rh1 occurs in 20 % and on the other, that rhrh amounts to 15 %, to calculate the absolute occurrence of the 3 genes. (This calculation has been omitted, as it would lead us too far in detail). By means of these percentages TAYLOR and RACE could ascertain the distribution of the various genotypes among men, which is recorded in Table I.

Table I.

Phaenotype	Genotype	Percentage
sub type Rh1	Rh1 Rh1	20
	Rh1 Rh2	14.4
	Rh1 rh	35.1
sub type Rh2	Rh2 Rh2	2.6
	Rh2 rh	12.5
rh negative	rh rh	15

During their investigations RACE and his collaborators detected a serum of a woman that like the serum of WIENER reacted exclusively with the gene Rh1, *viz.*, in 70 % (in Table I this accounts to 69.5 % *viz.*, 20 % + 14.4 % + 35.1 %). Thereupon followed the detection of the serum of a woman, which induced agglutination in merely 30 % and thus clearly merely acted upon the gene Rh2 (See Table I 14.4 % + 2.6 % + 12.5 % = 29.5 %).

Starting from the anti-Rh serum used thus far and from the latter three new sera, *viz.*, St serum, anti-Rh1 and anti Rh2 serum the latter scientists were able to ascertain not merely the phaenotype, but in nearly all cases the genotype as well, a result not yet attained at for other blood groupings. This is expressed in Table II.

[1]) The existence of different Rhesus factors makes it possible, that also Rh positive mothers may obtain an agglutinin acting on another factor than they possess themselves. Such cases, however, seem to be rare.

Table II

Genotype	Anti Rh serum	Anti Rh1 serum	Anti Rh2 serum	St serum
Rh1 Rh1	+	+	—	—
Rh1 Rh2	+	+	+	+
Rh1 rh	+	+	—	+
Rh2 Rh2	+	—	+	+
Rh2 rh	+	—	+	+
rh rh	—	—	—	+

Merely the types Rh2Rh2 and Rh2rh may not be distinguished by these means; all the remaining genotypes show a difference in the various reactions.

Next to the Rh genes mentioned as yet WIENER, RACE, CAPPEL and FARLANE traced other be it less frequently occurring genes, *viz.*, Rh′, Rh″, Rh_0 in 1 % and Rh_y in a still smaller percentage which all reacted as well with one or the other of the mentioned Rh sera. A further difficulty in the testing of womens sera is caused by the circumstance, that these sera in a few cases appeared to contain not one, but several of the mentioned anti-Rh agglutinins. So the combinations anti-Rh1 + anti-Rh2; anti-Rh1 + St; and even anti-Rh1 + Rh2 + St were met with.

The greatest difficulty in the Rhesus investigation, however, is caused by the fact, that the deviating sera cannot be prepared at will, but that the obtaining of them has to be left to chance. The more material is sent in for investigation and the greater the experience attained at is, the greater the chance will be to detect such sera. And, moreover, the disposal of a great number of persons the Rhesus state of which is known, will be needed.

I will thus conclude by presenting as my opinion that in order to attain the highest degree of exactness in these investigations the cooperation of all workers in this field is essential.

Literature.

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ON BACTERIOPHAGES AGAINST PLAGUE, OCCURRING IN CANAL WATER AND SEWAGE IN THE NETHERLANDS AND ON THE ORIGIN OF THESE PHAGES

by

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D'HÉRELLE is of the opinion that merely one kind of phage exists. This would normally parasitise on *Bacterium coli*. This is the reason why he added to the name *Protomicrobe bacteriophagum*, which he coined for the phage, the adjective *intestinale*. According to him the phage has initially merely lytic potencies against *Bacterium coli*. In contact with other, more especially pathogenic, micro-organisms the phage may develop the lytic property which it possesses potentially against such a microbe and may become a phage of the latter. According to him all known phages have once been phages against *Bacterium coli*. Sometimes the affinity for *Bacterium coli* has been preserved; in other cases, however, it may be completely lost.

The number of scientists, who share this conception with D'HÉRELLE is merely small. Fairly generally it is assumed that a multitude of phages exists. Still the scientist, who studies phages against pathogenic and more especially intestinal bacteria, again and again hits on phenomena which seem to substantiate the assumption of D'HÉRELLE. Among this kind of phages often strains may be detected which develop a wide range of virulence.

Sometimes these phage-sensitive bacteria belong to related species, sometimes not. Sometimes *Bacterium coli* belongs to the species against which the potency along with that against the specific phage-sensitive microbe has developed. D'HÉRELLE isolated a.o. a phage against the Gram-positive *Staphylococcus*, which acted as well on *Bacterium coli* and similar observations are reported by other investigators.

Difficulties are always met with, when the finding of an explanation for the presence of a phage with a virulence against a pathogenic germ which does not occur in the region where the phage has been isolated, is attempted at.

According to the conception of D'HÉRELLE the detection of a phage for instance against *Vibrio cholerae* or *Pasteurella pestis*,

either in human or animal discharge, or in the surrounding medium, might be considered as proof, or at least as a nearly certain indication that cholera or plague actually occur. To substantiate this assumption he emphasises that it is of no use to look for a bacteriophage against cholera in sewage, when no cholera occurs on the spot of the investigation. To illustrate this he reports the observation of AVERY in the Bacteriological Institute at Bombay. The latter looked during some months in vain for phages in the sewage at Bombay. This city was free from cholera at that moment. In June 1927 twelve cases of cholera occurred. Four days after the stating of these cases the test for cholera phages in the sewage was positive

D'HÉRELLE (6, p. 25) emphasises that under definite conditions the test for bacteriophages in the sewage may be the best method to hit upon an infectious disease in a community.

In regions where no cholera occurred, however, bacteriophages against cholera have been detected. As early as 1930 this has been reported by SÈGRE (8), who in the water of the river Po next to active bacteriophages against *B. coli*, *B. typhosum*, *B. dysenteriae* Shiga and Flexner detected also a bacteriophage against *Vibrio cholerae*. SCHLOSSMANN (7) as well found in surface water in Esthonia in the absence of cholera next to bacteriophages against *B. typhosum*, *B. paratyphosum*, *B. dysenteriae* Shiga and Flexner also one against cholera.

The water in the canals in the cities in the Netherlands, which generally are strongly polluted with human and animal feces, is as a rule rich in phages. This is in large measure the case for the water of the „Doelengracht" at Leiden, which passes the artillery barracks. During the whole year, but more especially in summer, phages against *B. dysenteriae* Shiga and plague may be always hit upon. Phages against plague were not merely detected in the canal water of Leiden but also in that of other cities (The Hague, Rotterdam, Dordrecht) and in the sewage of The Hague and Leiden.

The investigation, the results of which will be presented after a survey of the properties of the plague phages isolated at Leiden in 1926, aimed at the answering of the following questions:

a. Against which species of bacteria may phages be isolated out of the „Doelengracht"?

b. In which mode occur the phages in the water? As such or enclosed in cells of phage producers?

c. Of what origin are the phages against plague bacteria occurring in canal water?

THE PROPERTIES OF PLAGUE BACTERIOPHAGES.

As early as in 1926 bacteriophages against plague have been isolated by one of us (2) in the water of the „Doelengracht". The phage Doelen A was active against plague bacteria in the dilution 10^{-8} and dissolved also *B. coli* and *B. dysenteriae* Shiga in dilutions of respectively 10^{-6} and 10^{-7}.

After having been transmitted more than 20 times either on plague bacteria, or on *B. coli* or on *B. dysenteriae* Shiga, it preserved this range of virulence. After these transfers it kept moreover another property, *viz.*, the property to lose after one hour's heating at 56° C. the ability to lyse *B. coli* and *B. dysenteriae* Shiga, whilst retaining the ability to lyse plague bacteria. Had the heated phage been transplanted some times on plague bacteria, it lysed again like a non heated phage *B. coli* and *B. dysenteriae* Shiga.

In a later paper some other phages isolated out of the „Doelengracht" have been described. These as well were virulent against *B. coli* and *B. dysenteriae* Shiga and behaved similarly when they had been heated at 56° C. In this paper the method was described which was followed for the isolation of the phage. This consisted out of a mixing of 100 ml canal water with a same amount of nutrient broth, an inoculation of the mixture with a young culture of plague or other bacteria and an incubation during 16 hours at 37° C. Subsequently the suspension was filtered through a filterpaper, through which previously a not too concentrated suspension of kieselguhr had passed and the filtrate was tested as to its lytic action on plague bacteria. It was notable that none of the plague phages was obtained out of the suspension of plague bacteria in water. This was merely accidental, as later this actually has been the case, but during this investigation for instance the phages A and C were obtained after inoculation of the water with *Bacterium coli*; phage B after inoculation with *Bacterium dysenteriae* Flexner; phage E after infection with *Vibrio cholerae* (Flu (3,4)).

Six years later in 1934 this subject has been taken up again. The phages had been cultivated since their isolation in 1926 and 1927 uninterruptedly on plague bacteria and were tested in1934 after they had been transplanted more than 200 times in plague bacteria. It appeared that phage A had retained best its lytic property against *B. dysenteriae* Shiga. It lysed completely all of the 6 strains of *B. dysenteriae* Shiga which we used in the testing of its lytic action. Phage B acted merely on one strain, whilst phage C lysed not a single one. After rapidly consecutive passages through plague bacteria all strains reobtained the ability to lyse *B. dysenteriae* Shiga. When the phage strain was left unfiltered during a certain time the lytic action against *B. dysenteriae* Shiga was lost again, reoccurring, however, after some passages through plague bacteria. Whilst after a passage through plague bacteria the unfiltered phage lysed *B. dysenteriae* Shiga, the same phage when filtered by means of Chamberland or Berkefeld candles or by Seitz membranes, however, was inactivated against *B. dysenteriae* Shiga.

It could be established with certainty that each individual phage particle possessed the above properties and this feature was considered as pleading for the individuality of the phage.

In 1936 the plague phages A, B and C were tested once more.

None of them lysed a definite strain of *B. dysenteriae* Shiga (151), but they did so after some passages through plague bacteria. These Shiga-lysing strains were transmitted now through plague bacteria as well as through *B. dysenteriae* Shiga. After the 10th passage phage C, transferred through *B. dysenteriae* Shiga lost the property of lysing plague bacteria, *B. dysenteriae* Shiga, however, was lysed.

The filtrates of all passages had been stored in the ice-box. An attempt to reproduce the phenomena by starting anew from the 5th passage and transferring regularly through plague and *B. dysenteriae* Shiga failed however. By unknown cause a property of the phage had got lost. Just as the phage may reobtain its lytic action on *B. dysenteriae* Shiga, it can lose its lytic action on plague bacteria, but whilst the former function may be reactivated at will, the loss of the lytic action against plague may not be roused at random and it appears impossible to make the phage reobtain its lost potency.

In 1939 Dr E. A. Wolff in our laboratory succeeded to isolate plague phages out of the „Doelengracht", from sewage and from canals at Leiden. The potencies of the phages detected by Wolff may be read in the following table.

Table I.

Potencies of the phages isolated by Wolff.

Titer after the	Phage from sewage in Leiden against		Phage from Rapenburg against		Phage from Doelengracht against		Phage from Wetering, in which sewage is let in, against	
	P. pestis	*B. dysenteriae* Shiga	*P. pestis*	*B. dysenteriae* Shiga	*P. pestis*	*B. dysenteriae* Shiga	*P. pestis*	*B. dysenteriae* Shiga
6th passage via *P. pestis*	10^{-10}	10^{-2}	10^{-8}	10^{-7}	10^{-8}	10^{-8}	10^{-5}	10^{-7}
10th passage via *P. pestis*	10^{-9}	trace of lysis	10^{-7}	10^{-6}	10^{-9}	10^{-6}	10^{-7}	10^{-7}
20th passage via *B. dysenteriae* Shiga	10^{-7}	10^{-7}	10^{-8}	10^{-9}	10^{-8}	10^{-9}	10^{-8}	10^{-8}

Also in this investigation it became apparent that the potencies of the isolated plague phages after passages through micro-organisms belonging to various species remain constant and even in the most unfavourable cases deviate in merely a small degree from those of the original strain.

H. Flu, during 1939—1940, continued the examination of the „Doelengracht" for plague bacteriophages. In numerous cases

phages against plague bacteria were isolated. Six of them had a strongly lytic action and were studied more closely. Two acted merely on plague bacteria and not on *B. dysenteriae* Shiga and *B. coli*. Four acted next to on plague bacteria also on *B. dysenteriae* Shiga. One of these four moreover on *B. coli*. Among the four phages which directly after their isolation were virulent against several species, 3 appeared to consist out of mixtures of phages against plague, *B. dysenteriae* Shiga and *B. coli*. Merely one out of the isolated phages retained their range of virulence, independently of a transmission through *P. pestis*, *B. dysenteriae* Shiga or *B. coli*.

These series of observations continued since 1926 learn that, without the occurrence of plague, it is possible in any season of the year to isolate phages from canal water and sewage, which sometimes act solely on plague but often are also virulent on *B. dysenteriae* Shiga and *B. coli*.

Besides phages against *P. pestis*, *B. dysenteriae* Shiga and *B. coli* phages against numerous other microbes may be isolated out of the „Doelengracht". Sometimes the variation in phages occurring in one and the same water sample is great. Thus in March 1940 from a single water sample phages against the following bacteria have been isolated: *B. coli*, *P. pestis*, *B. dysenteriae* Shiga, *B. dysenteriae* Flexner, *B. dysenteriae* Strong, *B. dysenteriae* Sonne, *B. dysenteriae* Schmidt, *B. dysenteriae abdomalis*, *B. paratyphosum* A, *B. paratyphosum* B, *B. enteritides* Gärtner, *Corynebacterium diphtheriae*, *Bac. subtilis*, *B. faecalis alcaligenes*, *Bac. megatherium*, *P. pseudotuberculosis* (*B. pseudotuberculosis rodentium*), and a Gram-positive spore-forming small *Bacillus*, which has not been identified.

Investigations of one of us (5) have shown that water, either distilled or in a salt solution of low concentration is not a suitable medium for the phage, nor is „Doelengracht" water or river water, for during storage of such waters in tanks its content of phages decreases strongly.

We have tried to answer the question in what mode the phage occurs in the water. This might be as a free particle. With feces and other excreta the phage particles might occur in the water and be conserved in it during some period, or develop at the cost of other susceptible bacteria occurring in the water. The phage might as well get into the water along with lysogenic bacteria and be set free when these organisms perished. Finally it might be conceived that water bacteria produced the phage along with their development.

It seemed quite simple to solve this problem. It might suffice to filter a definite amount of water in such a way that a bacteriologically sterile filtrate is obtained and examine the latter.

It appeared, however, in parallel tests often that 0.01 ml of the water examined by the means as described on page 197 would contain phages e.g. against *B. dysenteriae* Shiga, whilst out of the water filtered directly, thus without any previous culture, through Chamberland L_3, 1, 5, 10, 20 and even 50 ml would produce negative results.

Especially striking were the results of the examination of soil samples for phage against *Bacillus megatherium*. When the samples had been mixed with broth and kept over night at 37° C., sometimes in 100 % of the tested samples phage might be detected. When, however, the water in which the soil was suspended was filtered through Chamberland L_3, than the result of the examination of this filtrate was nearly always negative, even when 10 and 50 ml were tested. Sometimes the filtrate of a dense suspension of soil through an ordinary filter paper gave a negative test. We arrived at similar results when horse feces was examined as to the occurrence of bacteriophage against *B. dysenteriae* Shiga.

It would be presumptuous to conclude from these results that the phage does not occur freely in the liquids investigated, but is contained in the bacteria which are held back by the filter. Investigations carried out by one of us (5), which have shown that during filtration of bacteriophages in protein-free salt solutions the phages may be completely adsorbed by the substance of the filter, plead for prudence. Addition of small volumes of protein containing liquids, such as for instance broth, to the filtrate, prevents the adsorption by the filter. The content of proteins in canal water is not nought, but at all events too smal to shield the phages against adsorption, whilst the content of salts is high enough to promote the adsorption.

It appeared in fact in a parallel investigation, that whilst the filtrates of the unmixed liquids mentioned before appeared to be free from phage, the filtrate appeared to contain phages and sometimes in large numbers, when before the filtration the liquid had been mixed with a same volume of nutrient broth, or when before the filtration of the liquids nutrient broth had been made to pass the filter.

Thus it was certain that phages occur as such in the canal water and also in soil.

It is, however, possible that next to the free phages lysogenic strains might occur as well in the latter media. In view of this possibility more than 1500 germs isolated out of water were tested for their lysogenicity. In a centrifuge which made 9000 turns per minute and the tubes of which could contain 40 ml of liquid a volume of the canal water was centrifugated during half an hour. A thin film of suspensa was formed, which contained most of the bacteria occurring in the water. The liquid was poured off carefully, the tubes filled once again and again centrifugated. This was repeated 5 ×, by which means after the final centrifugating 800 ml liquid had been centrifugated at 9000 turns per minute.

Now the depot was distributed over a large number of agar plates, the separate colonies streaked on agar and tested as to their lysogenecity against *B. dysenteriae* Shiga. Among the 1500 colonies tested merely one appeared to possess the faculty to produce, when grown with *B. dysenteriae* Shiga in broth, phage against *B. dysenteriae* Shiga. This lysogenic strain appeared to be *Bacterium coli*.

In this investigation none of the species belonging to the so-called water bacteria could be isolated as „producing phage against Shiga".

So it has been proven that next to the free phage particles occurring in water lysogenic Shiga-phage producing bacteria may be detected. In the discussion about the origin of the phages occurring in water these lysogenic strains will be mentioned again.

As may be seen in the list on page 199, during the ascertainment of phages occurring in a sample of canal water also a phage against *Pasteurella pseudotuberculosis* (*Bacterium pseudotuberculosis rodentium*) could be detected. *P. pseudotuberculosis* possesses an antigenic structure which agrees with *P. pestis*. When a phage lyses *P. pseudotuberculosis* it is probable that *P. pestis* may be lysed as well by this phage, just as a phage against *B. dysenteriae* may lyse as well *Bacterium dysenteriae* Shiga, *Bacterium dysenteriae* Flexner or Y bacteria.

The problem of the occurrence of a bacteriophage against plague in the canal water of Leiden, whilst plague was an unknown disease in the Netherlands, might thus seem to be solved.

In fact D'HÉRELLE claimed to have solved the problem in this way. In his book (6) published in 1938 he mentions on p. 58 to have noted, that all bacteriophages against *P. pestis* isolated by him were active as well against *P. pseudotuberculosis* and against other *Pasteurella*. He supposes that the plague phage isolated by one of us will be active against *P. pseudotuberculosis*. He claims to have confirmed this supposition by means of tests on phages received from FLU in 1938. According to him the plague phage isolated by one of us out of canal water would be actually a phage against *P. pseudotuberculosis* and thus the paradox of the occurrence of plague phages in the Netherlands without the occurrence of bubonic plague would be explained.

An investigation into the lytic potencies of the isolated phage against a number of strains of plague bacteria in our collection made it apparent that by the phage against *P. pseudotuberculosis* out of the 26 strains studied 19 were lysed strongly, 2 feebly and 5 not. It appeared moreover that all bacteriophages against plague isolated by WOLFF and by H. FLU would lyse *P. pseudotuberculosis*. Very remarkable was the behaviour of the phages A, B and C isolated in 1926. They behave similarly against *P. pseudotuberculosis* as against *B. dysenteriae* Shiga. When the phages were tested after

a lapse of more than a week after the transmission through the plague bacteria, they lysed merely plague bacteria. Neither *B. dysenteriae* Shiga nor *P. pseudotuberculosis* were acted upon. When the unfiltered lysate of plague bacteria by the phages of A, B and C was made to act upon *B. dysenteriae* Shiga or on *P. pseudotuberculosis*, a complete lysis occurred. *P. pseudotuberculosis* behaved in exactly the same way as *B. dysenteriae* Shiga, as is shown in Table II.

Table II.

The lysis of *Bacterium dysenteriae* Shiga, *P. pestis* and *P. pseudotuberculosis* by some bacteriophages against plague.

Phage	*P. pestis*	*P. pseudo-tuberculosis*	*B. dysenteriae* Shiga
A	++++	+++	++++
B	++++	+	+
C	++++	++	++

++++ = complete lysis, +++ = nearly complete lysis, ++ = feeble lysis, + = trace of lysis.

The virulence against Shiga bacteria could be increased by some rapidly succeeding inoculations in Shiga and filtration, but this method failed for *P. pseudotuberculosis*. On the contrary the phage got lost and after 3 transmissions through *P. pseudotuberculosis* the filtrate was without the least action on this organism.

If lysates of *P. pestis* by the phages A, B and C and kept during a longer period without filtering are added to young cultures of *P. pestis* and *P. pseudotuberculosis*, which are both present in a same tube with broth, complete lysis of both bacteria will occur.

It appears that the phages A, B and C actually posses some potency against *P. pseudotuberculosis*, lose this potency during a longer storage, reobtain the lytic potency as a fresh lysate through plague bacteria and cannot be made to increase in virulence against *P. pseudotuberculosis*, in contradistinction to their behaviour when transmitted through *B. dysenteriae* Shiga. So the problem is less simple than it is claimed by D'HÉRELLE.

The phage against *P. pseudotuberculosis* has been transmitted 19 times through a lysable strain of *P. pestis*. Each time 0.1 ml of the filtrate was added to 10 ml broth. The final dilution after the 19th transmission was 10^{-38} of the initial phage.

In a same way the phage was transmitted 26 times through *P. pseudotuberculosis*. Here the final dilution was 10^{-52} of the initial phage. The lytic action of these filtrates was compared with the action of the initial phage and the following results were arrived at.

Table III.

Comparison of the action of bacteriophage *P. pseudotuberculosis* before and after transmission.

	On *P. pseudo-tuberculosis*	On *P. pestis*
Directly after isolation	10^{-8}	10^{-2}
After 19 transmissions through *P. pestis*	10^{-10}	10^{-5}
After 26 transmissions through *P. pseudotuberculosis*	10^{-8}	10^{-3}

The results reported in Table III do not substantiate the assumption of D'HÉRELLE, that the phages against plague detected by us are actually phages against *P. pseudotuberculosis*. On the contrary it is prudent to assume that we are working with two different phages which may develop potencies more or less to either side.

Before we arrived at this conclusion, we had looked for the origin of phages against *P. pseudotuberculosis* diligently but with negative results. It is self-evident that the discharge of rats and guinea-pigs had to be tested as to its occurrence. The results of a testing of the discharge of 15 white rats, 15 guinea-pigs and 23 rabbits for such a phage were completely negative. Neither in the discharge of poultry, ducks and horses the phage was detected nor in 500 germs cultured out of water and tested as to their lysogenicity against *P. pseudotuberculosis*. The testing of the discharge of rats, guinea-pigs and rabbits was not only negative as to phage against *P. pseudotuberculosis* but as well against Shiga and plague.

The results, however, were otherwise when the investigation was extended over wild grey rats. These animals could be furnished by farmers out of the neighbourhood of Leiden. They had been slain to death, so that we could merely collect the content of rectum and coecum.

20 wild grey rats were examined. In 3 animals the test for phages was negative. In 2 phages were detected against *P. pestis*, *B. dysenteriae* Shiga and *P. pseudotuberculosis*. In 8 as well against *P. pestis* as against *Bacterium dysenteriae* Shiga. In 4 merely against *P. pestis*. In 2 merely against *B. dysenteriae* Shiga. In 1 merely against *P. pseudotuberculosis*.

In 12 out of 20 rats a phage against *P. pestis* and in 3 out of these 20 rats a phage against *P. pseudotuberculosis* could thus be detected.

By streaking the intestinal content of rats with positive plague and pseudotuberculosis phage, bacterial strains were obtained which were tested as to their lysogenicity by plague and pseudotuberculosis phage. Out of one of the animals which was a carrier

of plague and pseudotuberculosis phages, 3 lysogenic strains were obtained. These strains belonged to the coli group.

No *P. pseudotuberculosis* could be isolated out of the intestins of any of these rats and neither could any injury of the organs characteristic for *P. pseudotuberculosis* be noted.

The properties of the strains producing phage against Shiga isolated out of canal water and those producing phage against plague isolated out of the intestins of the rat are taken up in Table IV. For the sake of comparison the properties of *P. pseudotuberculosis* which has been used in our investigation are taken up as well.

Table IV.

Behaviour of the phage-producing strains on some media.

Name of the microbe	Milk litmus whey	Production of indole	Glucose meat infusion	Glucose glutamine	Lactose meat infusion	Lactose glutamine	Maltose glutamine	Saccharose glutamine
plague phage producing strain 10	curd, reduction	+	gas	acid	gas	acid	acid	acid
plague phage producing strain 2	curd, production of acid	+	gas	acid	gas	acid	acid	acid
plague phage producing strain 31	curd, production of acid	+	gas	acid	gas	acid	acid	acid
Shiga phage producing strain 43	curd, production of acid	+	gas	acid	gas	acid	acid	acid
P. pseudo-tuberculosis	no curd, blue	—	—	acid	—	—	trace of acid	—

The bacteria were moreover Gram-negative and did not liquefy gelatine. They belonged to the coli group. It had still to be ascertained whether they were merely infected with phage or actually lysogenic.

Bordet and Renaux (1) have shown for *B. coli* which produced phage against Shiga, that in oxalate containing media in which calcium salts are precipitated in insoluble form, lysogenic strains may be cultivated during a long period with successive passage through oxalate containing media without losing the potency of lysogenicity. Strains which are merely mixed with the phage lose after passage through oxalate meat infusion the potency to produce phage during their development.

The pure lysogenic strains were inoculated in oxalate meat

infusion. They developed well therein. On four consecutive days they were inoculated in fresh oxalate meat infusion broth. On the 5th day they were inoculated from the 4th transmission through the oxalate broth to ordinary meat infusion which was filtered then for the test for phage. Along with these transmissions through oxalate broth the strains were inoculated in ordinary meat infusion broth, so that strains which had been transmitted four times through ordinary meat infusion broth were available for the test for phage. The testing of the various culture media for phage gave the following results.

Table V

Influence of the passage through ordinary and oxalate meat infusion broth on the production of phage.

Name of the strain	Ordinary meat infusion				Meat infusion oxalate
	1st passage	2nd passage	3rd passage	4th passage	5th passage
Shiga phage producing strain 43	—	—	—	+	+
plague phage producing strain 2	+	+	+	+	+
plague phage producing strain 31	+	—	—	—	—
plague phage producing strain 10	+	—	—	—	—

— = no phage, + = phage.

It can be learned from Table V, that the strains of *B. coli* producing phage against Shiga and those producing phage against plague are actually lysogenic, but that the other strains were mixtures of phage and *B. coli*.

It is now possible to answer the question about the origin of phages against plague in sewage and canal water. Phages against *P. pestis* and lysogenic *B. coli* which produce that plague phage were detected in the intestins of a large percentage of grey rats. Rats are regular visitors of canals and sewers. They may be seen at dusk running along the canals or cross them swimming.

Our investigation does not furnish a single argument for the assumption that the „plague phages" would actually be phages against *P. pseudotuberculosis*. On the contrary everything pleads for the fact that these phages are coli phages which accidentally possess potencies against plague and *P. pseudotuberculosis*. This is also the case for the phage against Shiga which is produced by

the lysogenic coli. Plague phages as well as the Shiga phage had in fact a lytic action against *B. coli*.

Summary.

The properties of a number of bacteriophages against plague, which have been isolated during the period of 1926—1940 from sewage and canal water have been described.

These phages show a wide range of virulence. Nearly always next to plague bacteria *B. coli* and *B. dysenteriae* Shiga are lysed as well.

Also phages which are doubtlessly pure retain the virulence against several species and other typical properties, which may be noted already in the impure phages.

The behaviour of the pure phages against plague, A, B and C, isolated in 1926 out of canal water was studied more closely. These strains were always transmitted through plague bacteria and displayed directly after their isolation the phenomenon that by heating at 56° C. during an hour they had lost the property to lyse *B. coli* and *B. dysenteriae* Shiga, but had retained this action against *P. pestis*. By a passage through *P. pestis* the lost properties came back.

Later the latter property got lost completely and another was discovered which up till the moment remained constant. After a longer storage and by filtration through a L3 filter after a single passage the phage loses the virulence against *B. coli* and Shiga bacteria and recovers this merely after repeated rapid passages through plague bacteria.

In the course of the investigation it could be stated that without any obvious reason the phage may lose some of its virulences, even that against plague bacteria.

The phage occurs in the canal water as free phage, but also enclosed in lysogenic *B. coli*.

In order to detect the free phage the water has to be mixed with a same volume of nutrient broth as otherwise out of even 50 ml water all phages will be adsorbed by the filter (Chamberland L3).

The reason of the contamination of canal water and sewage by bacteriophages against phage has to be looked for in the pollution of such liquids by the discharge of wild grey rats (*Mus decumanus*). In the intestins of more than 70 % of these animals a phage against plague was detected and merely 5 % of them contained a phage against *P. pseudotuberculosis*.

Our results cannot substantiate the assumption of D'HÉRELLE, that the „plague bacteriophages" isolated by us would be actually phages against *P. pseudotuberculosis*. It is true, however, that plague bacteriophages may lyse *P. pseudotuberculosis* and phages against pseudotuberculosis may lyse plague bacteria. Clear-cut differences, however, may be stated between both phages.

In our opinion the bacteriophages against plague as well as those against *P. pseudotuberculosis* and *B. dysenteriae* Shiga which have been detected in the canal water, have been phages against *B. coli*.

Not merely pleads herefor that they are produced during the development of lysogenic *B. coli*, but also the fact that they have often retained a potency against *B. coli*. The fact that some of these phages miss the potency does not plead against this assumption as during our investigation some bacteriophages against plague which directly after their isolation possessed such a potency have lost this in the course of the investigation.

Nothing pleads against the assumption that a phage against plague, *B. dysenteriae* Shiga or *P. pseudotuberculosis* which does not possess this potency may have possessed it in an earlier stage of its existence.

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ABSTRACTS

J. A. Keverling Buisman, Enkele nieuwe aminosulfonamiden en aminosulfonen en hun „Sulfonamide-activiteit". (Some new aminosulphonamides and aminosulphones and their „sulphonamide-activity"). Thesis, Groningen 1946.

A number of compounds was synthesised and tested on sulphonamide-activity. By this is meant a bacteriostatic action in vitro, which is annihilated by 4-aminobenzoic acid (vitamin H'). The bacteriostatic activity of a compound was measured by the time, in which a culture of pneumococci in ascites-broth, in presence of the compound, becomes opaque, in comparison with a control test. In case of bacteriostasis the influence of extra-added 4-aminobenzoic acid was studied in a separate test; when normal growth occurred in this last test the compound possessed sulphonamide-activity. The investigated compounds belong to three groups:

I. Compounds derived from sulphonilamide and its N'-derivatives by substituting the benzene nucleus by the pyridine and thiazole nucleus (2-aminopyridine-5-sulphonamide and 2-aminothiazole-5-sulphonamide). These compounds do not possess sulphonamide-activity.

II. Compounds derived from 4,4'-diamidophenylsulphone by substituting one aminophenyl group by a phenyl, 2-pyridyl or 2-thiazyl group. These groups do possess sulphonamide-activity. The compounds in which said group is substituted by 2-quinolyl, 2-benzthiazyl, 1-naphthyl and 4-amino-1-naphthyl are too weakly soluble to be examined.

III. 4-aminophenyl-2'-pyridylsulphide does not possess sulphonamide-activity.

Ty. E. Galesloot, Over de vroeg beginnende gasvorming in kaas. (On the gas formation occurring at early date in cheese). Thesis, Wageningen 1946.

The investigation has been confined to the coli-aerogenes bacteria. A great many strains have been isolated; in a combined fermentation and curdling test in milk coli types and some intermediate types although able to ferment lactose with the production of acid and gas, under the conditions of this experiment did not produce any gas. Neither were they able to produce a considerable amount of gas from whey. By means of the same test in synthetic skim milk, which did not contain any citric acid, it could be proved that it is this acid in milk which prevents the latter types from producing gas in the above test. The types which do not produce any gas could

even prevent the production of gas by the other types. By means of this test the strains have been subdivided in blowing-positive and blowing-negative ones. A simple fermentation test in yeast-water-lactose proves that a supply of citric acid prevents the production of gas from lactose by the blowing-negative types. After the acid has disappeared gas is again produced. The citric acid evidently acts as a hydrogen acceptor and presumably via oxalic, malic and fumaric acids, is reduced to succinic acid. As a result the blowing-negative types decompose the sugar present in such a way, that no gas is produced. The blowing-positive types, however, do not use citric acid in a similar way. On the contrary the addition of this acid increases the gas production from lactose by these types, for shortly after the beginning of the fermentation, they take to fermenting the citric acid as well, giving an additional gas production.

When a larger amount of this acid has been supplied, the blowing-negative bacteria do not use all of it in the changed mode of decomposition of the lactose, but they also will take to fermenting part of it, so that an increase in gas production occurs. This conclusion was drawn from the fact that testing these strains in yeast-water-lactose with 0.05 % citric acid, the beginning of gas production was postponed for about the same lapse of time as when 0.20 % had been given, and that the gas production was sometimes higher after a supply of 0.20 % than of 0.10 %. Moreover coli-bacteria which had developed for some time in a liquid containing lactose and citrate, such as whey, were able to grow in KOSER's citrate solution.

In experiments with cheese it appeared that the types which could not induce the phenomenon of blowing in the fermentation-curdling test, neither could do so in cheese. Thus the blowing in cheese can as a rule be merely caused by the aerogenes-cloacae types and by some intermediate ones. Moreover the blowing caused by blowing-positive types decreased when negative types were also present. In cheese the blowing-negative types kept alive much longer than the others. Evidence could be furnished that the citric acid in these cheese tests acted in the same way as in the experiments with liquid cultures.

By means of fermentation tests it could be ascertained that KNO_3, KNO_2 and $KClO_3$ prevent gas production by blowing-positive types, whilst $KBrO_3$ and $K_2S_2O_8$ do not. The way in which these substances inhibit gas formation was studied more closely. KNO_3 appeared to be reduced, first to nitrite, subsequently to hydroxylamine. As long as the bacteria convert sugar in the culture solution, this hydroxylamine combines with ketoacids, produced in this decomposition, forming oximinoacids. The latter compounds, after a reduction to aminoacids, are assimilated. When no further decomposition occurs, the remaining hydroxylamine is reduced to ammonia. During the latter process the bacteria, which are not damaged by previous reduction processes, die off. It is due to these

reductions in which hydrogen derived of the sugar dissimilation is used, that the bacteria are unable to produce much gas from sugar. KNO_2 acts in the same way as does KNO_3; $KClO_3$, however, appeared to prevent gas production by the blowing-positive types much more efficiently, being thereby reduced probably to KCl. Next to its action as a hydrogen acceptor, $KClO_3$ also inhibited gas production, because, along with the reduction intermediate products occur which prevent the development of bacteria. As soon as these reduction processes have come to a close the production of gas starts.

By means of adding these salts to the milk the blowing phenomenon in the cheese made therefrom could be checked. Nitrate, nitrite or chlorate had to be added in such amounts that the gas production from lactose was put off by the occurring reduction processes until the moment in which gas production could be sufficiently checked by the lactic acid bacteria. 2 g of $KClO_3$ added to 100 l of milk acted as strongly as 50—80 g KNO_3 or KNO_2. When the latter substance had been supplied an unfavourable increase in pH occurred, as the nitrite strongly impedes the lactic acid fermentation. As the development of the coli-aerogenes bacteria in cheese was not hampered by the supply of KNO_3 or KNO_2 we could not by these means combate the fermentation taste caused by these bacteria. As far as we could ascertain, the use of $KClO_3$ does not cause any harm, as the compounds which are formed during the reduction of $KClO_3$ are very toxic to coli-aerogenes bacteria, the fermentation taste caused by these bacteria could be successfully prevented by means of this salt. The lactic acid fermentation, however, was not impeded by this reduction. $KClO_3$ is recommended as a successful means of preventing the blowing of cheese. Under practical conditions the addition of 1 g to 100 l of milk may prove sufficient.